Odour Perception and Orientation in Fishes

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INTRODUCTION

As is true of so many aspects of structure and function in the fishes, the olfactory mechanism occurs in a multiplicity of variations, for here is a group of animals whose span of existence in geological time is so extensive as to have permitted endless opportunities for adaptation to many types of aquatic environment, with their associated biotic and community odours. In addition, mutations favouring olfactory acuities have had great periods in which their survival value could be tested.

Odour perception in fishes will be interpreted here as those sensations, chemically induced, which are relayed to the central nervous system from the olfactory sac. Taste, and what is termed the common chemical sense, will be referred to as sensations perceived by gustatory nerve endings in the mouth and over the entire body.

In humans, substances that are tasted are interpreted as being sweet, sour, bitter, alkaline. To be odorous to man a substance apparently must fulfil two conditions: It must be volatile at ordinary temperature and must be soluble in fat solvents. All the known odour substances either are gases or have a high vapour pressure, boiling below about 300°C. Most inorganic substances exhibit very low vapour pressure and have no discernible odour. A few have unpleasant odours such as the halogens (fluorine, chlorine, iodine, bromine), phosphorus, ozone and certain compounds such as hydrogen sulphide, sulphur dioxide, nitrogen oxides and ammonia.

For organic compounds the situation is very different; they are much more

likely to be odiferous and their odours have a vastly greater range. Of nearly half a million synthetic compounds listed in encyclopaedias of organic substances, a large proportion have a high enough vapour pressure to be detected in the nose.

Numerous experiments show that substances to which insects respond at one concentration may repel at a higher concentration (Prosser *et al.*, 1950). Substances which are repellent at low concentration usually elicit a more violent repellent response at higher concentrations. These changes in response suggest the participation of a new group of receptors, perhaps those mediating the common chemical sense at these high concentrations.

A smellable substance must pass into solution on the mucous film to be perceived by a terrestrial vertebrate; therefore, the view most generally accepted is that olfaction in all animals is aquatic in the final sense, and this will be acknowledged here with no further comment.

We are concerned here with a sense of such refined acuity that it defies comparable attainment by the most sensitive instruments of modern chemical analysis. Man can detect the odour of methyl mercaptan at an approximate concentration of 9×10^{-13} M, which is 10,000 times more dilute than the minimum concentration of an active taste stimulant such as strychnine hydrochloride. Moncrieff (1944) comments: "Man is microsomatic, yet how wonderful his sense of smell which detects quantities far beyond the limits of balances, microscopes, or other laboratory paraphernalia, with the possible exception of the spectroscope." In insects, only a few molecules in a liter must be present in the turbulent air being carried from a female moth to attract a mate from two miles distance. Odour thresholds for dogs are not known accurately. Certainly, the sense of smell is most acute in hunting breeds; and the ability to perceive the sex odour is notoriously keen.

One modern theory of odour perception (Kistiakowsky, 1950) suggests that odoriferous substances act by interfering with enzyme-catalysed reactions in the receptors. Since enzymes are affected in their action by minute amounts of a variety of substances, this theory plausibly explains the high sensitivity of human sense of smell and the wide range of compounds that possess odours. The rapid reversibility of inhibitory effects on enzymes would account for the speedy adaptation of the reception system enabling it to register new odour impressions.

Classification of odorous substances have been anthropomorphic for the most part (Moncrieff, 1944). What may be odorous to man may not necessarily be so for fish. Therefore, at this stage in our knowledge, little relation exists between types of sensation inducing physiological or behaviouristic activity and the chemical nature of the stimulant.

Odour perception is often referred to as a distance perceptor, as in the case of emanations of a chemical in which a gradient of concentration exists to its source. Not only is its acuity greater than taste perception, but this sense is responsive to multifold substances, most of which are organic in nature.

In fishes, we are dealing with an acuity of olfaction which matches any attainment of terrestrial animals.

ANATOMY OF THE OLFACTORY ORGANS IN FISHES

Among the fishes, extreme variations can be found in the morphology of olfactory organs. In the sharks and rays the paired olfactory pits are usually situated on the ventral side of the snout. The single opening of each pit is more or less divided by a fold of skin into an anterior inlet and a posterior outlet, the latter sometimes leading into the mouth. As the fish swims through the water, and particularly as it takes water into its mouth in breathing, a current of water is passed through each of its olfactory sacs. In this way the olfactory organs are influenced by the respiratory current. In the lungfishes the anterior apertures are external, whereas the posterior openings lie within the mouth and correspond to the choanae of higher vertebrates.

In the bony fishes, their paired olfactory pits are almost always on the dorso-anterior aspect of the head and are in no direct way connected with the respiratory current (Figure 1). In nearly all fishes each pit has two entirely separate openings, an anterior inlet and a posterior outlet. By means of these two openings, a current of water enters and leaves each pit.

Burne (1909) described the olfactory organs of 51 genera of fishes comprising 32 families. Fundamentally, he observed that water may be brought into play upon the laminae of the rosette in three ways:

1. By action of cilia within the anterior nostril and upon the lining membrane of the olfactory chamber (eel).

2. By deflection of water into the nose-cavity during forward progression. This deflection may be effected by the position and slope of wide-open nostrils to the horizontal, as in *Esox*, but more frequently it is brought about by a hood or screen upstanding behind the anterior nostril (Gadids, carp). In this case the force of the current is under the control of the fish, and varies directly with the pace at which it is moving. In noses of this type a further
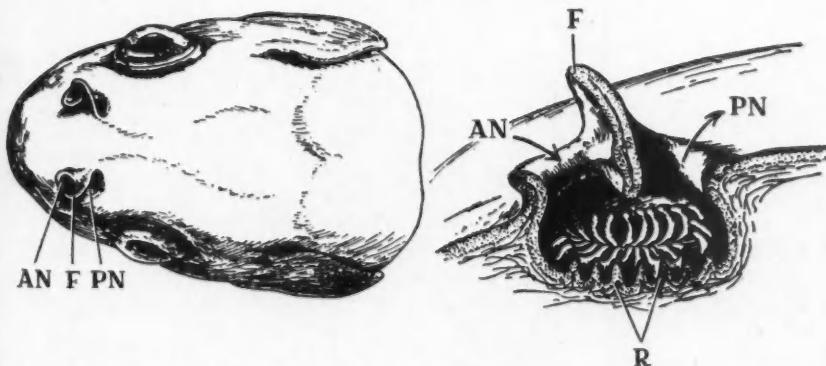

FIGURE 1. Position of nares and dissection of olfactory capsule of the minnow, *Phoxinus*. After von Frisch (*Die Naturwissenschaften*).

AN = Anterior naris.   PN = Posterior naris.   F = flap.   R = olfactory rosette.

refinement is frequently met with in the form of an internal flap that conducts the water current right down into the centre of the rosette.

3. By alternate dilatation and compression of accessory sacs connected usually with the hinder part of the olfactory chamber.

In the majority of cases these are acted upon by the movements of the pre-maxillae and maxillae, occasionally (*Capros, Clupea*) by those of the mandible, or (Siluroids) of the palatine bar, and in several cases (where the lachrymal sac extends upon the buccal membrane) by the general pressure of the water in the mouth during expiration.

The currents produced normally by the sacs are rhythmical, flowing in and out of the nose as the fish gently opens and closes its mouth in breathing. The strength of the current must, however, be quite under the control of the fish, for sudden and energetic movements of the jaws would naturally produce corresponding sudden and strong currents in the nose, comparable to a sniff. In noses of this class it is generally arranged by means of valves that the water shall enter by the anterior nostril and leave by the posterior.

Classified according to habitat, Burne believed the fish could be grouped in three categories on the basis of olfactory organs: (1) bottom fishes, (2) slug-gish shallow-water fishes, and (3) free-swimming open-water fishes.

Pipping (1927) has classified olfactory mechanisms into four general types:

1. Where water is deflected through a capsule as the fish moves through the water. (*Esox lucius, Nerophis ophidion, Siphonostoma typhle* and *Spheroides maculatus.*)

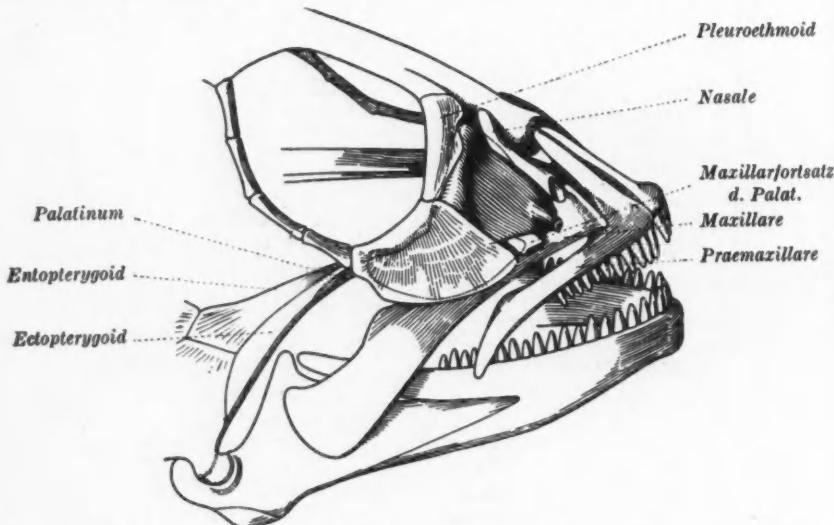2. Where breathing movements compress an olfactory pouch to draw water

FIGURE 2. Sketch of the skull of the Blenny, *Zoarces viviparus,* showing the pouch-like nose which operates like a syringe. After Liermann, 1933 (Verlag G. Springer).

in and out of a single opening. (*Cottus scorpius, Zoarces viviparus, Gasterosteus pungitius, Gasterosteus aculeatus* and *Rhombus maximus*; see Figure 2.)

3. Where water moves in one opening and out another owing to breathing movements or to the action of cilia during pauses in breathing. (*Cottus gobio* and *Pleuronectes flesus*; see Figure 3.)

4. Where water circulates in through the anterior aperture and out the posterior, whether caused by breathing movements, cilia or both. (*Leuciscus rutilus, Leuciscus erythrophthalmus, Phoxinus aphya, Perca fluviatilis, Acerina cernua, Gobius niger*—Figures 4, 5 and 6.)

An unusual anatomical form was described in *Zeus faber*, the John Dory, by Berghe (1929). In this fish the olfactory rosette is suspended by a fold in the nasal cavity. Respiratory movements compress surrounding lymphatic sinuses which in turn act upon the nasal cavity in such a way as to force the rosette against the anterior naris, blocking and opening it alternately like a valve. Ducts connect these sinuses with other lymphatic sinuses of the head.

Pipping (1927) observed flow of water in and out of the nasal pit of a nonmoving Plötze (*Leuciscus rutilus*). Cilia draw water through the anterior opening and propel it out of the posterior opening. In the perch (*Perca fluviatilis*) the nasal pouch is operated when surrounding bones (Figure 4) compress it during respiratory movements. Liermann (1933) and Pipping noted that carmine particles were taken in the anterior opening and expelled through the posterior.
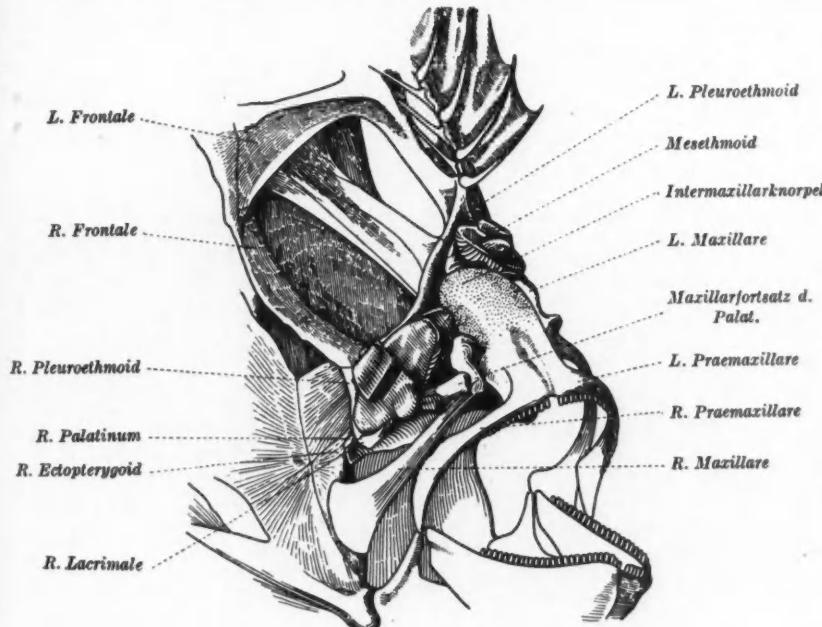

FIGURE 3. Skull of a flounder, *Pleuronectes flesus*, with the olfactory organs sketched in. After Liermann, 1933 (Verlag G. Springer).
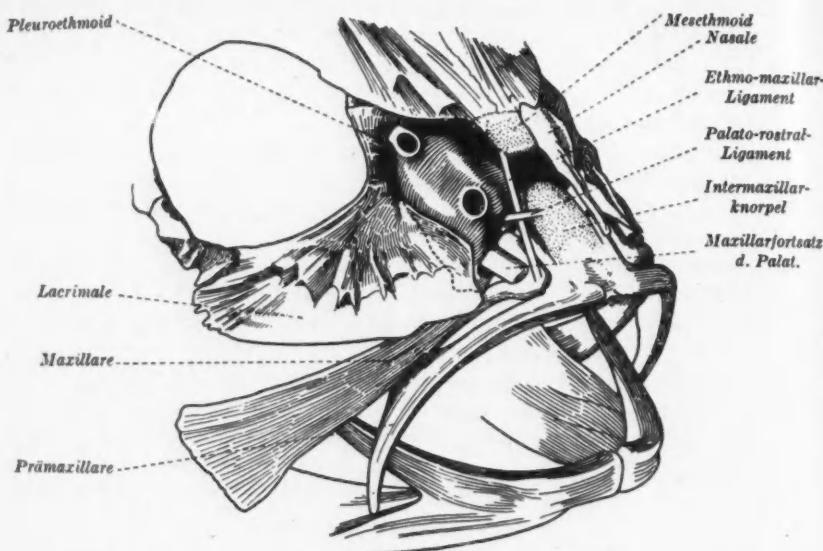
FIGURE 4. Skull of the perch *Perca fluviatilis*, with the olfactory organ emphasized. After Liermann, 1933 (Verlag G. Springer).
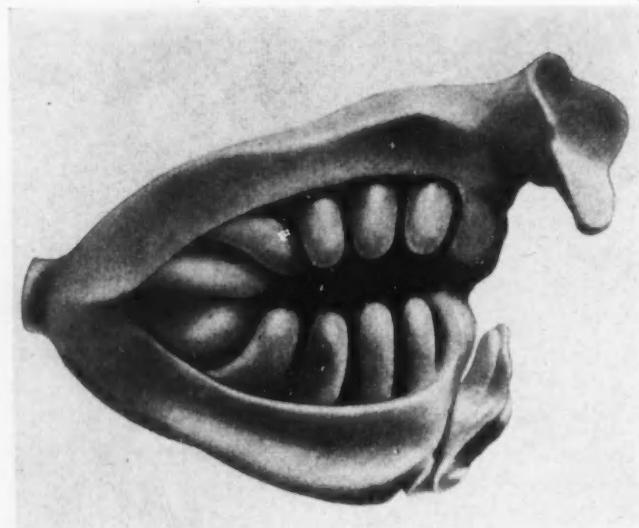

FIGURE 5. A model of the olfactory rosette of the perch (×32). After Liermann.

It appears to be a very weak suction, but unidirectional none the less. Both Pipping (1927) and Wunder (1927) established positive odour perception in the European perch, but there was much variation observed in the use that various individuals made of it in food-getting.

The eel (*Anguilla vulgaris*) has a very elaborate development of nasal apparatus (Figure 6). It is elongated, with many folds extending laterally like veins in a leaf. Ciliary action alone is responsible for flow of water over many folds of the epithelium according to Pipping and Liermann.
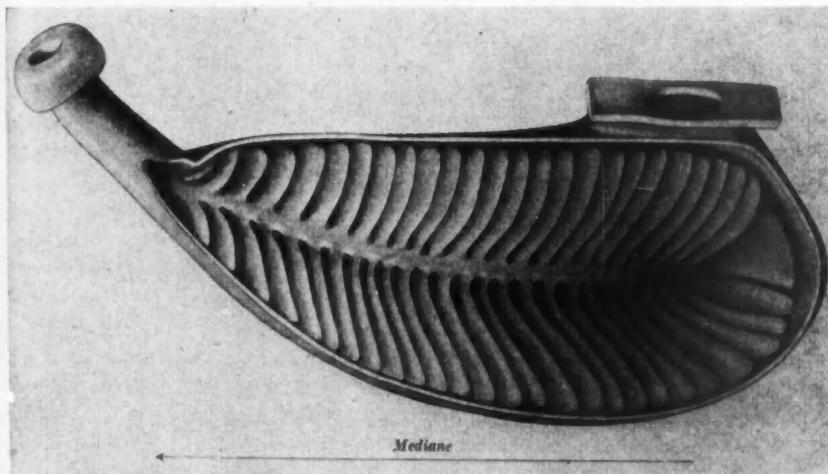

FIGURE 6. Model of olfactory rosette of the eel ($\times 20$). After Liermann.

A specialized structure with enlarged surface area, such as this, obviously allows many more sensory cells to be activated.

The histological structure has not been described in many species, although Adrian and Ludwig (1938) have pictured the ciliary nature of the epithelium, and Blaue (1884) shows olfactory buds lying alongside stratified epithelial cells. Jagodowski (1901) described sensory hairs in *Esox lucius* whose distal ends reach through the olfactory mucus, but this observation needs verification with modern techniques.

Wunder (1936) believes that the morphologically best adapted "nose" is one that renews water rapidly and completely. Fish with a one-holed pouch for sampling the environment are not the best smellers.

The stickleback (*Gasterosteus aculeatus*) has a poorly developed nasal capsule. Both Pipping and Wunder have shown experimentally that it has an inferior scenting capacity that plays no rôle in food-getting, thus conforming to its simple anatomy. Not only is it a simple sac-like structure, but also only the small upper two folds bear sensory receptors.

To a modern reviewer, the paucity of precise anatomical and histological

descriptions for common North American species is a surprising void in the literature. Many student projects in universities could be profitably devoted to such descriptions.

Gross observations of the anatomy of *Oncorhynchus kisutch*, the coho or silver salmon, by the author would classify it in Pipping's group I, although histological sections may later show the epithelium to be ciliated. Certainly, the main flow of water over the olfactory tissues is produced by swimming movements or by currents in the stream. But here also, no observations have been made to determine how breathing movements influence the flow of water.

IMPULSES FROM OLFACTORY TISSUE TO BRAIN

In certain fishes (bullhead, carp and tench) the olfactory bulb is connected with the forebrain by a nerve strand (the olfactory stalk) (Figure 7), which measures about 2 cm. long and is composed of from 500 to 1,000 medullated fibres. Adrian and Ludwig (1938) studied the action potentials in the olfactory stalk of the bullhead during stimulation of the olfactory end-organs. Potential changes of small amplitude passed up the stalk as long as the preparation survived, though nothing but distilled water had been introduced into the sac. This resting discharge was of very low frequency. When the sac was irrigated with fluid containing small fragments of some odorous material, e.g. putrefying earthworms, a burst of impulses occurred at high frequency after a latent period of from 0.5 to 5 seconds or longer. It was found that the irrigation was more powerfully stimulating if the fluid contained small fragments of the material than if it had been filtered. Mechanical stimulation, caused by pressing upon the roof of the sac, or by touching its interior with a brush, was also found effective. The nervous discharge was not rhythmical, there being no evidence that groups of fibres discharged synchronously.

TASTE

The distribution of taste-buds in the vertebrate classes indicates the presence of this sense in the mouth regions in forms as low as the amphibians. Reviewing the literature for fishes, Herrick (1903) lists over 35 species in which taste-buds are known to occur on the outer surface of the animal as well as in the mouth. Among them are the carp, sturgeon, cod, ling, mullet and catfish. The bullhead *Ameiurus* is remarkable in this respect in that its whole outer surface is provided with these organs, which are most abundant on the barbels. When a piece of meat is brought into contact with the barbel of one of these fishes, the animal will immediately seize and swallow the morsel. The same is true when the meat is brought in contact with the side of the fish. This quick seizure and swallowing of the food has been called by Herrick the gustatory response. The same form of response is made to meat-juice discharged from a pipette on the side of the fish. That these responses are really gustatory is shown by the fact that when the branch of the seventh nerve that innervates the taste-buds on the flank of *Ameiurus* is cut, the responses no longer occur (Parker, 1912). The anatomy of the gustatory elements in fishes is briefly discussed by Evans (1940).
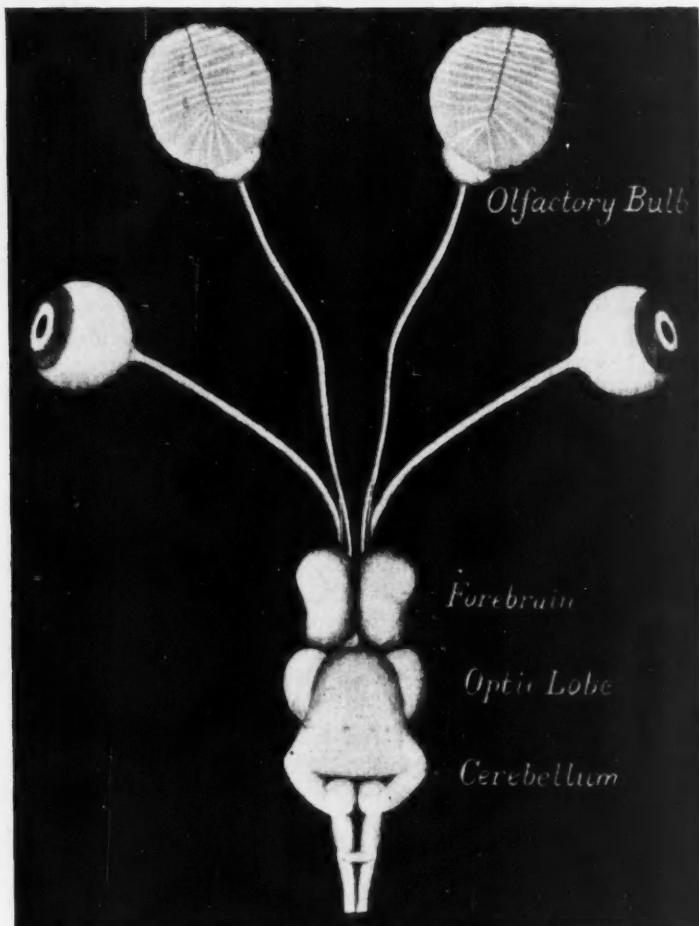
FIGURE 7. The olfactory bulb of the bullhead (*Ameiurus*) and its relation to the forebrain. After Adrian and Ludwig (1938).

Conditions similar to those in *Ameiurus* were recorded by Herrick in a number of other fishes, and it is thus clear that taste is a general integumentary function in many of these animals.

Nagel's (1894) observations on Amphioxus were confirmed by Parker in 1908, when it was shown that the skin of this lower chordate was sensitive to solutions of acids, alkalis, alcohol, ether, chloroform, turpentine, oil of bergamot and oil of rosemary, but not to solutions of sugar. It was also demonstrated that the skin of *Ameiurus* was sensitive to sour, saline and alkaline solutions, a condition that was subsequently found to be true for the young of the lamprey (Parker, 1908, 1912).

Early works on food acceptance as related to taste by Aronsohn (1884), Steiner (1888) and Baglioni (1909) are of little consequence to this review. Sheldon (1909, 1911) demonstrated in the dogfish that the sensitiveness of the nostrils of this fish to weak solutions of oil of cloves, pennyroyal, thyme and the like, was not influenced by severing the olfactory crura, but disappeared on cutting the combined maxillary and mandibular branches of the trigeminal nerve. Evidently the nasal surfaces of this species, like those of the higher vertebrates, are innervated by fibres from the trigeminal nerve.

These observations warrant the general conclusion that the outer surfaces of most fishes and amphibians are receptive to stimulation by chemical substances of a mildly irritating kind.

Wunder (1927, 1936) studied the perception of natural foods by taste-buds located on the body, barbels, lips and gill-rakers. He described in particular a large, well-polstered pad on the roof of the carp's mouth, whose entire surface is strewn with taste-buds enabling it to take in miscellaneous items, from which it appears able to sort the food organisms from detritus. Northern pike (*Esox lucius*) were also tested by Wunder, who reports taste receptors only on the inside of the mouth. Earthworms soaked in alcohol and subsequently washed in water were rejected by a blind, de-olfacted fish when it took the worm into its mouth; the same extracted worm was accepted if dipped in fresh worm juice.

Wunder believes that northern pike and carp make little use of their noses, which function only for food-getting when the fish are swimming, whereas his blinded trout were able to locate food by olfaction at considerable distances. His carp with plugged nostrils were not handicapped in food-getting under laboratory conditions.

The Bartgrundel (*Nemachilus barbatulus*) lost its ability to perceive tastes on its flank receptor when Dijkgraaf (1934) severed the innervating branch of the *N. facialis* (Figure 8). The action of acids on taste receptors in the mouth of *Phoxinus* was studied by Klenk (1930). In 1935, Scharrer described sensory cells in the free fin-ray of *Trigla*, which perceived acids and sardine extract; also he and others (1947) reported on taste responses in two marine fishes.

Strieck (1924), in von Frisch's laboratory, removed the forebrain of the minnow, *Phoxinus laevis* and initiated a training regime on specimens that had learned to anticipate food ,when presented with cotton wads soaked in sugar solution, but no food if given salty, bitter or sour substances. Such an olfactory-excised fish, when trained, would energetically search the bottom for food each time the training taste was presented on a cotton wad, and ignored any other taste. He established thresholds of 10 to 11 per cent for grape sugar, 6 per cent for salt (NaCl), 0.15 per cent for acetic acid and 0.02 per cent for quinine. Later, Trudel (1929) attempted to discern if other tastes that are sweetish for man are also detected by fish. For negative training, he used punishment such as presenting broken glass in the cotton wad, applying a blow with a glass rod, or electric shock. He found that rapping on the aquarium also produced the desired effect when a negative taste was presented. Trudel taught his minnows to discriminate between several natural sugars. They even responded to saccharin.
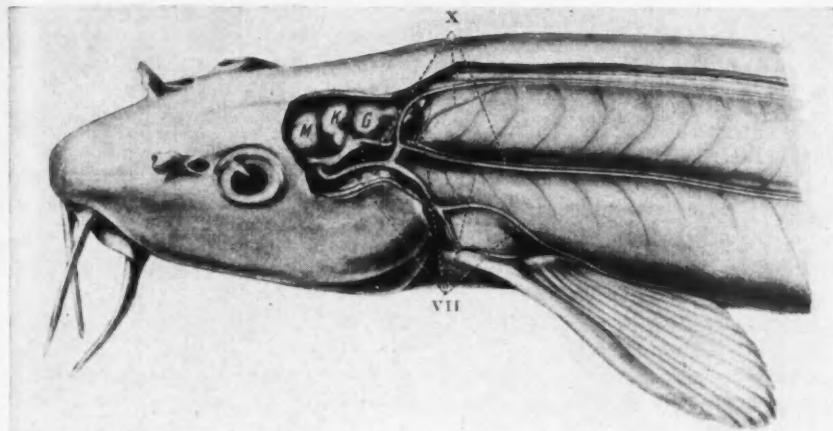
FIGURE 8. Dissection of X and VII cranial nerves of *Nemachilus,* which supply the nerve endings of the taste and common chemical sense system. After Dijkgraaf, 1934 (Verlag G. Springer).

Quinine was perceived as low as 0.0025 per cent, whereas his human subject had a threshold of 0.0003 per cent.

This differential conditioned-response test gives more decisive and critical proof of the ability of the animal to discriminate between odorous materials than did the spontaneous responses recorded by earlier workers.

A shark deterrent, copper acetate, was recommended by Burden (1945); this substance is irritable, no doubt, to the common chemical sense. This work needs reverification under a more rigid experimental design. Our laboratory preliminary tests showed it to be toxic but not repellent to salmon. Whiteley and Payne (1947) have also tested shark repellents.

Following this work, Krinner's (1935) trained minnows detected NaCl at dilutions of 4×10^{-5} M and saccharose at 2×10^{-5} M. He found their sugar thresholds to be 512 times lower in concentration than those for man; and their salt thresholds 184 times lower. Removal of olfactory tissues did not change these thresholds. Therefore, a true gustatory sense was verified. Hunger motivation and long practice were essential features of Krinner's training discipline.

OLFACTION IN FISHES—PERCEPTION OF FOOD ODOURS

Animals such as dogs have elaborately developed olfactory organs; man, a poor scenter, has less than most land mammals. More pertinent to our theme, the whale, which has gone aquatic, has only vestigial olfactory organs; there has been no readaptation to olfaction in water.

As among the higher vertebrates, fishes, except cyclostomes, possess paired olfactory sacs varying greatly in complexity of form and function. The innervation is essentially identical with the corresponding parts in the air-breathing verte-

brates. Nevertheless currents of water flow through these sacs, and such stimulation as they receive must come from these currents.

As early as 1895, von Uexküll observed that dogfishes from which the olfactory membranes had been removed did not respond to the presence of food, whereas normal dogfishes searched eagerly three to five minutes after food had been introduced into their tank. Subsequently, he soaked their food in quinine, an odourless, bitter-tasting substance. It was expectorated as soon as taken into the mouth. Bateson (1889), after describing the anatomy of several olfactory capsules of fishes, said that the rockling, with olfactory organs extirpated, did not notice food and concluded that these organs served only when in direct contact with food.

In these experiments no attempts were made to exclude sight or to ascertain the effects of the operation. In experiments carried out by Parker (1910), an attempt was made to gain more conclusive evidence. Five normal bullheads (*Ameiurus*) were allowed to swim in an aquarium in which were hung two wads of cheesecloth, one of which contained concealed earthworms. In the course of an hour the wad containing the worms was seized 11 times by the fishes, even though it was periodically interchanged in position with the other. During the same period the wad without worms was passed over by the fishes many times and never excited any noticeable reaction.

Ten bullheads were next prepared for further experimentation; in five of these the olfactory tracts were severed, and from the remaining five the barbels, the seat of the chief external gustatory organs, were removed. After the fishes had recovered from these operations, they were put in an aquarium into which was introduced a wad of cheesecloth containing minced earthworms. During the first hour, the wad was seized 34 times by fishes without barbels but with normal olfactory organs; whereas, although fishes with cut olfactory tracts often passed over the wad, it was never seized by any, and was "nosed" only once by one of them. None of these fishes paid any attention to a wad of cloth containing no worms. Repetitions of these tests by Parker gave similar results, and led to the conclusion that the olfactory organs of the bullhead are serviceable in sensing food at a distance much beyond that at which the organs of taste are capable of acting; in other words, bullheads truly scent their food.

Recently in our laboratory, a student project, using a technique discussed later in this review, established the ability of the minnow *Hyborhynchus* to discriminate between three species of aquatic invertebrates, *Gammarus fasciatus*, a species of caddis fly (*Hesperophylax* sp.), and *Hyalella*.

Parker (1911a) excluded the olfactory organs of *Fundulus* by stitching up the anterior nares. As a result of this operation the fish no longer responded to hidden food, but quickly reacquired this power after the anterior nares had been reopened. These results were confirmed in work on the dogfish, *Mustelus*, by Sheldon (1911) and on the swellfish, *Spheroides*, by Copeland (1912). Sheldon closed the nares of the dogfish with cotton plugs and, in 1914, Parker showed that when only one nostril is thus plugged, the fishes turned persistently toward the side of the open nostril. Such responses indicate that, in the seeking of food

under normal conditions, dogfishes, and probably other fishes as well, turn toward the side on which the concentration of odorous particles is greater. In criticism of this conclusion, it has been pointed out that the animal may as likely have turned *away* from the irritating plug as *toward* the open nostril. A good example of an animal in which this condition reaches its extreme is the hammerhead shark in which the nostrils, as well as the eyes, are carried on the remarkable lateral projections that extend sideways from its head.

Parker (1911b) mentions that sharks can be drawn from a long distance by ill-smelling bait or by oily fish carcasses ground up and thrown into the water as in the practice of chumming.

In testing various natural substances, Olmsted (1918) observed, "But since human saliva proved fairly stimulating to the fish (bullhead), the time-honored custom of 'spitting on one's bait' does seem to be more than superstition, and perhaps for this reason may receive the sanction of science." Using 30 pairs of fish at different seasons and charting the number of bites by two fish during half-hour trials and grading the most active material on the basis of 100, he found earthworms high, liver next and human saliva third.

Olmsted sewed the nostrils of a trained bullhead and observed that it did not respond to a bag of earthworms until it touched it with the barbels. In further tests he allowed that odorous materials which stimulate bullheads are possibly of a protein nature and are not of the nature of fats or volatile oils. He believed that these proteins were present as chemical traces and could not be detected by ordinary qualitative tests for proteins.

ENVIRONMENTAL ODOUR PERCEPTION

A many-sided rôle may be ascribed to the sense of smell over and above food-getting. Wrede (1932) has stressed the part it plays in aggregating schools of fish, notably the Elritze (*Phoxinus*). These minnows are attracted by a substance in the mucus of that species—individuals congregated in that part of the aquarium where mucus from other minnows was introduced. Furthermore, once assembled, they appeared less restless. Both blinded and hind-brain-extirpated fish could be trained to these substances, indicating chemo-reception, although animals with nose intact responded best. Perhaps, Wrede postulated, optical stimuli by day and odour by night are the signals important in aggregation over the 24-hour period.

Wrede built a simple three-compartment maze. A specimen was placed in one of two small sections for a few minutes, then removed. A test animal, introduced into an adjoining compartment could sniff at two apertures and enter either. It was only fed if it entered the positive section and soon learned to associate the odour of a different species with the correct compartment.

Following up this suggestion presented by Wrede, Göz (1941) designed a testing apparatus (Figure 9), which made possible conditioned-response experiments to show the Elritze's ability to discriminate, by odour, 15 different species of fish representing eight families. (Positive: reward by feeding; negative: punishment by a light blow from a glass rod.) Precautions were taken against
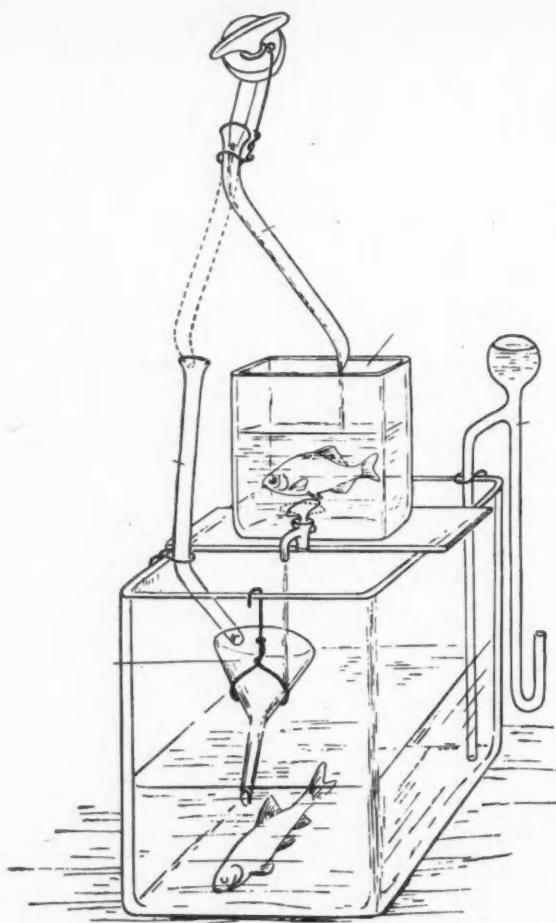
FIGURE 9. Arrangement used to train fishes to give a feeding reaction when exposed to the odour of another species. After Göz, 1941 (Verlag G. Springer).

collateral cues. By positive and negative training, his fish learned the difference between closely related species within its family and could discriminate a training odour even if it was mixed with those of other species. Göz claimed to have shown further that individuals within a school have an individual body odour recognizable by each member of the school.

A fright reaction was elicited in the Elritze by odours from *Perca fluviatilis*, *Salmo fario* and *Ameiurus nebulosus*.

Blinded minnows, not reacting to pike odour, were placed in with a pike who captured some of them. The remaining were again tested and showed that a combination pike and Elritze odour induced a fright reaction. Thus the negative

reaction of the minnow to *Perca fluviatilis, Salmo fario* and *Ameiurus nebulosus* may have been the result of traumatic experiences with these three species. This may not be, therefore, a test of innate response.

Strong fright reactions were observed by Noble (1939) in young *Tilapia* to some substance released from injured fish skin, but no quantitative data were given; he merely mentioned the fact in passing in a general article on animal behaviour.

In a methodical series of experiments, von Frisch (1941) examined the nature of a substance he calls an alarm substance (Schreckstoff). When an injured minnow (*Phoxinus laevis*) was placed among its school which had been accustomed to a feeding platform, the same school would exhibit an alarm reaction ("Schreckreaktion"). The school would concentrate after a latent period of 0.5 minutes and immediately seek cover or flee. The alarm reaction is incited by a repellent given off by the injured skin of *Phoxinus* (the Elritze). When 100 ml. of this solution was poured into aquaria of 25- to 150-litre capacity, positive reactions were recorded. Some variability was noted between different schools of fish; most satisfactory reactions were recorded when schools were kept in aquaria to which they had been conditioned for some time. No alarm substance was found in extracts of intestine or liver. The ovaries were only one-hundredth, muscle one-twentieth, and gills one-fifth to one-tenth as active as skin. When the olfactory lobe is destroyed or the olfactory nerve severed the minnows do not respond to the substance. Therefore, the alarm substance is odoriferous. Control fish showed that the operation, *per se*, was not a complicating factor. The skin of dead minnows retains its activity several days. Hüttel (1941) made some chemical tests of the alarm substance from Elritze skin. It appears to be a purin- or pterin-like substance.

The activity of extracts from 41 species of fresh-water fishes was tested and compared to those from the Elritze. Eighteen species were from other than the Cyprinidae. Their skins contain no appreciable quantities of the alarm substance for the Elritze. The skin extracts from the Cyprinidae were active in general, although only two of the 23 species tested approached the activity of Elritze skin. Relationship alone does not explain the variability of activity within the Cyprinidae. Perch (Percidae) do not exhibit an alarm reaction to their own skin extracts. *Squalius cephalus, Rodeus ararus* and *Scardinius erythrophthalmus* (all Cyprinidae) give an alarm reaction to extracts of skin of their own species. There appears to be a specificity of reaction in these species, because the reaction is strongest to extracts of the species tested. The reaction is augmented in the Elritze when water from the pike (*Esox lucius*) is mixed with skin extract of the Elritze. Experiments in nature show that the alarm response is associated with the location, because the minnows take food at a point some distance from the area of alarm, but behave nevertheless more nervously than when they are thoroughly conditioned. On two occasions this reaction was observed in nature, once when a school was attacked by perch and again when it was disturbed by a bittern. These experiments and observations aid in the understanding of the development and maintenance of schooling behaviour of non-predatory fishes,

because of the general survival advantage the group receives from an alarm or repellent substance given off by an injured comrade.

Recently, Brett and MacKinnon (1952) poured rinses of human hands into a salmon ladder during an autumn adult migration. These rinses proved surprisingly repellent to the ascending coho salmon. Control fluids, water, urine and tomato juice were ineffective,

Hiatt *et al.* (1953) have recently tested large numbers of artificial substances for their repellent threshold to fishes. Human skin irritants, lachrymators and nerve poisons are irritable to fishes. Multihalogenated organic compounds, organic thiocyanate and halogenated ketones disperse fish in schools, some in as low concentrations as 0.05 p.p.m. Their effect is not necessarily olfactory.

ODOURS OF AQUATIC PLANTS

The apparatus (Figure 10) used in the following citations from our laboratory consisted of several seven-gallon aquaria, each with a siphon-airlift circulation system installed in both ends. Water was siphoned from the aquarium, returned by air pressure, and discharged into a six-inch funnel which was suspended above the tank. The funnel was connected to a glass tube which lay across the end of the aquarium. Perforations in the tube directed the incoming water across the bottom of the aquarium. Water from the jet on one side flowed only about halfway across, because there it met the stream from the other end, and both were deflected upward. This produced two currents or convection cells, each of which involved one-half of the tank. Water samples containing the odours were introduced into the aquarium by means of a separatory funnel, which was connected to the siphon tube just after it left the tank. Two glass tubes, 2 by 24 inches in size, were filled with activated charcoal and connected in the circulation system of each tank. The water could be made to flow through them, during the interval between tests, without altering the rate of flow of the circulation. This treatment insured the complete removal of the test odours before the beginning of the next test and thus avoided olfactory adaptation of the fishes. It was found that trained fish did not respond to an odour after it had passed through the filter.

Two carbon electrodes about two inches apart were located in each end of the aquarium. Later a third one was installed three inches above the end. The space between them was designated as the "end zone," that is, the place where the fish were fed or punished depending on which odour was being introduced. By means of a switchboard, the operator could place 1.5 volts with 11.5 milliamperes of current across the electrodes at either end of the aquarium. The fish were rewarded by introducing food pressed on perforated celluloid strips into the end zone.

With a set of electrodes and a jet in each end of the aquarium, it was possible to train from either end when the order in which the scents were presented was randomized. In order to eliminate cueing toward the operator, the fish were blinded by chemical cautery (injection of phemerol into the posterior chamber of the eye). To remove any extraneous stimuli that the fish might receive during training the following precautions were taken: the aquaria were insulated from gross mechanical shocks by mounting them on sponge-rubber;
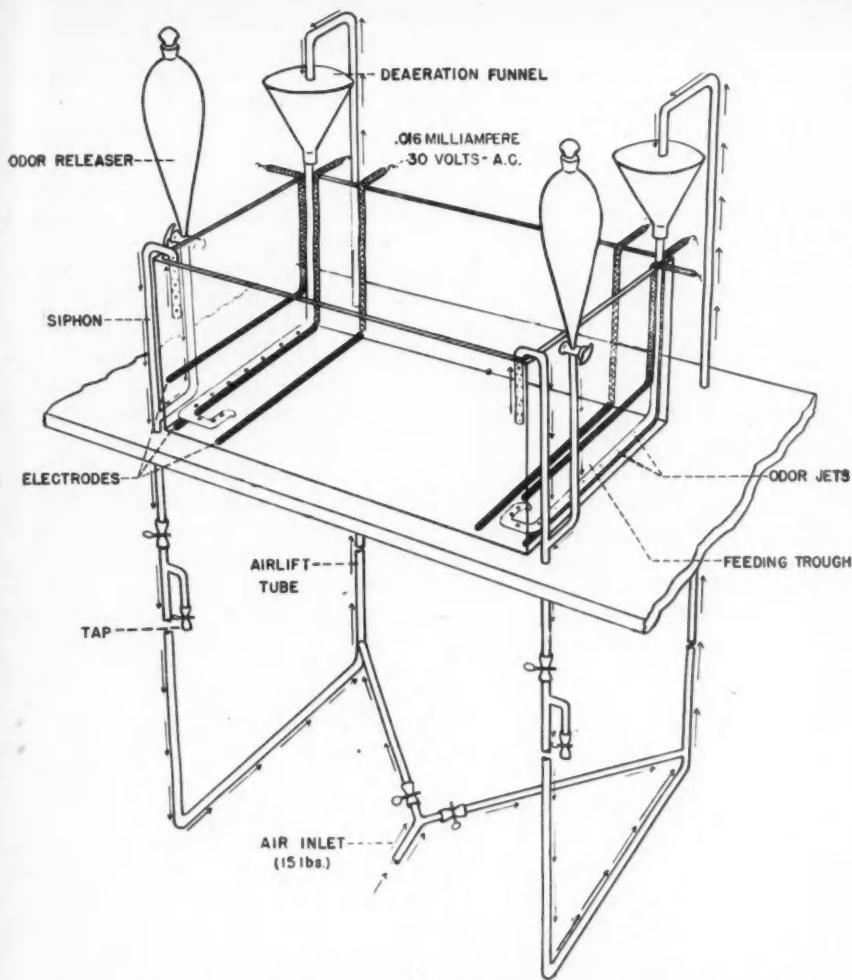
Figure 10. Apparatus for conditioning fish to discriminate odours. After Hasler and Wisby (1950).

introduction of the odour was accomplished in a manner that did not alter the dimensions of the stream of water which was constantly flowing into the aquarium; and the observer whispered scores to the recorder over a telephone system to avoid any association with change in pitch or rapidity of the observer's speech.

Walker and Hasler (1949) used this apparatus for quantitative recording of scores made by fish on a conditioned-response training regime, resulting in a new testing procedure giving reproducible results. They undertook to study the

odours of aquatic plants in the life of fishes. They showed that blinded blunt-nose minnows (*H. notatus*), in groups of six and eight, were able to discriminate, after a period of training of two and one-half months, between the odours of *Myriophyllum exalbescens* and *Ceratophyllum demersum*, when food was the reward and electric shock was the punishment. Reverse training substantiated this.

Similarly, these fish were trained to discriminate odours of other sets of plants: (I) *Ranunculus tricophyllus* and *Anacharis canadensis*; (II) *Utricularia vulgaris* var. *americana*; (III) *Potamogeton zosteriformis* and *P. crispus*; (IV) *P. amplifolius* and *P. vaginatus*. Rinses containing odours of other plants were introduced into aquaria of fishes so trained, in a Latin-square design by a generalization test. Of 12 species tested, the odours of only two seemed to resemble each other; that is, when *C. demersus* had negative significance, the fish associated with it the odour of *A. canadensis*. Trained minnows whose olfactory epithelium had been destroyed by heat cautery were unable to respond to plant rinses, thereby proving that the rinses contained a stimulating scent detected by sense of smell.

When minnows were fatigued by or adapted to a strong rinse of one species, they still detected odours of other species as they were superimposed. The minnows were able to detect a water rinse of a sprig of aquatic plant diluted 1:10,000 after it had been further diluted by streaming it into a seven-gallon aquarium, thus demonstrating a very high degree of odour-sensitivity.

The results of these studies lend support to the view that aquatic plants may well play an important rôle in the life of a fish. They may serve as signposts to guide fish into feeding grounds, since many fishes commonly feed in turbid water, at dusk, at dawn, and at night, when visibility is poor. Moreover, the odours of aquatic plants may serve as attractants to immature fishes in littoral areas of lakes to prevent them from straying from cover.

COMMERCIAL APPLICATION OF ODOUR PERCEPTION

Hasler and Wisby (1950) explored the application of their technique to biological assay of pollutants. Phenol, a common pollutant in municipal water supplies, cannot be readily detected in low concentrations until it has reacted with chlorine forming odorous chlorophenols. A method was presented in which bluntnose minnows (*Hyborhynchus notatus*) were used to detect the presence of phenols below the threshold for man (0.01 p.p.m.). Fishes of two aquaria were trained to associate the odour of phenol with food and the odour of a p-chlorophenol with punishment. The minnows of two other aquaria were trained to the same odours, but with reverse meanings. Upon completion of the training period, the minnows were discriminating successfully between these two substances at concentrations of at the most 5×10^{-4} p.p.m. A generalization test described by Hilgard and Marquis (1944), which was used to test o-chlorophenol, the third odour, indicated that the fishes were able to distinguish it from the two training odours. The biological assay of phenolic compounds by this method is primarily one of detection and not of quantitative analysis.

Phoxinus were trained by Neurath (1949) to descriminate eugenol and

phenylethylalcohol. Trained fish detected eugenol at dilutions of 17×10^{-6} p.p.m.; phenylethylalcohol at 23×10^{-6} p.p.m. He found the trained specimens were unable to discriminate these compounds after the olfactory lobes were extirpated. However, they would still respond to taste substances such as quinine and acetic acid.

Recently, Tester (1952) and van Weel (1952) have been examing the question of simulated bait attractants for tuna. Water extracts of tuna induced feeding reactions whereas extracts of white-fleshed species did not. It is still not clear if the fish had been inadvertently conditioned to tuna. The maintenance and testing of such large animals is fraught with difficulties.

ORIENTATION TO THE PARENT STREAM BY ODOUR

That salmon return, in the great majority of cases, to spawn in the stream or tributary in which they were reared, is acknowledged as fact by most modern fisheries biologists. Although previous workers who have investigated this phenomenon have advanced the idea that chemical stimuli may influence the movements of salmon, no one has been able to guide salmon to an artificial stream.

It has been postulated by Hasler and Wisby (1951) that there is, in river and creek water, some characteristic odour to which young salmon become conditioned while in the stream, and which they recognize and orient to upon reaching the parent stream as mature migrants. This theory embodies the principle that a salmon returning to its parent stream reacts differently to the odour of that stream than to any other. In order for a salmon to return to its home stream there must be the possibility of a differential reaction, not a simple response to a repellent or an attractant. This guiding odour must remain constant from year to year and have meaning only for those salmon which were conditioned to it during their fresh-water sojourn.

This theory presents three distinct problems:

(1) Do streams have characteristic odours to which fish can react? If so, what is the nature of the odour?

(2) Can salmon detect and discriminate between such odours if they do exist?

(3) Can salmon retain odour impressions from youth to maturity?

In order to answer the first question, a group of bluntnose minnows (fish of proven olfactory acuity), were trained to discriminate between the chemical differences of two Wisconsin creeks. That scent-perceiving organs were the sole means of discrimination in these tests was proven by destroying the olfactory tissue of trained fishes, after which they no longer responded to the training odours.

Chemical analysis of the stream waters indicated that the only major difference between them was in the total organic nitrogen fraction. Experimental evidence to substantiate this was obtained by separating the water into various fractions and then presenting these to trained fishes. The fish trained previously to natural water did not react to the inorganic ash, or to the distillate of residue of water fractionated at $100°C$. However, they recognized the distillate, but

not the residue, of water fractionated by vacuum distillation at 25°C.; a strong indication that the odorous stimulant is a volatile, aromatic substance.

A test was conducted of the retentive capacities of the trained minnow, and it was determined that even this fish, which is not specialized in this respect, could differentiate between the odours for a comparatively long period after cessation of training. Learned behaviour was found to be retained longer by young fish than by old.

The method of training, which was used with such success with the minnows, was then applied to salmon fry. After a short period of training it was evident that these fish too could discriminate between the odours of the two Wisconsin creeks.

It was proposed by Hasler and Wisby (1951) to employ an artificial substance to which salmon fry could be conditioned and which could then be used to decoy them, upon their return, into rehabilitated streams, formerly dammed or polluted, or to salvage a run which would not be able to pass a newly constructed power dam. Such an odour must be neither a repellent nor an attractant for unconditioned salmon.

Wisby and Hasler (1952) designed an apparatus to test the reactions of unconditioned salmon to various organic odours which might be used for the above purpose. This unit consists of four arms which converge on an enlarged central compartment. Water is introduced into the upper end of each arm and cascades down a series of eight small falls until it enters the centre compartment, from which it exits through a drain. The entrance to each of the four arms is guarded by a gate, all of which can be raised by pulling a single cord.

Salmon fry were placed in the centre compartment and, upon introduction of an odour into one of the arms, the gates were raised permitting the fish to enter the arms. Their distribution after the test was then noted and compared with the distribution obtained with no odour present.

Many organic odours were presented to unconditioned salmon, and their reactions noted. Of these, none was found which would attract salmon, many seemed not to be perceived, and the remainder were distinctly repellent in action at the concentrations used. Of the latter, only two were deemed suitable for further testing. One of these, dicyclopentadiene, proved to be so insoluble as to render accurate dilutions impossible.

It was observed that these fishes were not totally repelled by dilute solutions of morpholine, although it could easily be detected in concentrations as low as 1×10^{-6} p.p.m. Although the minimum detectable concentrations were not established, the concentrations presented indicate a sensitivity to chemical stimulation far exceeding that reported for any fish tested to date.

It appears, then, that the compound morpholine fits the requirements mentioned previously. It is soluble in water, thus permitting accurate dilutions; it is detected in extremely low concentrations, thus making the treatment of large volumes of water feasible; and, it is chemically stable under stream conditions. Furthermore, at these low concentrations, it is neither an attractant nor a repellent for unconditioned salmon and thus should have meaning only for those salmon previously conditioned to it. Field tests are now being conducted to determine

whether salmon fry and fingerlings which have been conditioned to morpholine can be decoyed to a stream other than that of their birth, upon their return to fresh water as mature migrants. An experiment of this kind to determine the nature of imprinting is of the highest importance relative to our hypothesis.

Craigie (1926) viewed the nasal cup as an aid to orientation in salmon. He cut the olfactory nerves of salmon captured at sea by a radical incision across the snout, but there were insufficient recaptures to show clearly a disrupted migratory path; also, the homing stream was believed to be the Fraser River, but was not known to be so.

Pursuing further this concept of odour recognition of a home stream, Wisby and Hasler (1953) captured sexually ripe coho salmon at two branches of the Issaquah River in Washington and returned them downstream below the fork to make the run and selection of stream again. In half of them the nasal sac was plugged with cotton. The great majority of normal fish selected again the stream of first choice, while the plugged-nose fish returned in nearly random fashion. No comparable pressure trauma was applied to the control fish; nevertheless, this experiment is indicative of the important rôle that the functional olfactory system has in orientation.

CONCLUDING COMMENT

Only intensive and imaginative studies in the future can show the subtle rôle of the sense of smell in fishes. The vast degree of morphological variation, the many different kinds of habitats, and the manifold community relationships present a wealth of experimental material and problems, the exploration of which can certainly advance our knowledge of olfaction.

In the area of orientation of homing fishes we need to answer the questions: Can fishes discriminate water masses sufficiently to stay within their boundaries? Can they "learn" several other stream odours besides the one of their parent river? Do specific points within a stream have characteristics "preferred" by olfactory discrimination? How are the cues from odours related, in biological meaning, to cues from sight and hearing? Are these cues that induce specific reactions meaningful only when they come in combinations or in succession?

Recent work in electrophysiology by Professor E. D. Adrian, Cambridge University, shows how chemicals in stimulating the olfactory tissues produce responses in different parts of the olfactory lobes. It is indeed pertinent to an understanding of olfaction. It opens up a new field of investigation which should be extended to fishes and correlated with their innate responses and learning.

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Hearing in Fish, with Special Reference to
Semotilus atromaculatus atromaculatus (Mitchill)[1]

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ABSTRACT

The literature on hearing in fish is reviewed. Experiments with *Semotilus a. atromaculatus* (Mitchill) showed perception over a range from 1 to 5,750 c.p.s. Fish from which the ears had been removed seemed to perceive frequencies from 20 to 200 c.p.s. Fish without the lateralis nerve behaved as normal fish. Normal fish could distinguish one-fifth of an octave in the range of 50 c.p.s. Trained on 50 c.p.s. they had absolute pitch for 70 c.p.s. A threshold curve over the range of 20 to 5,750 c.p.s. was established for this species.

Highest sensitivity was at 280 c.p.s.; lowest at 20 c.p.s. and above 2,000 c.p.s. The movements of the fish in response to vibration stimuli were studied by means of motion pictures. The fish were able to locate the source of vibration, most likely oriented by fields of higher intensity in the experimental tank. In approaching the source the fish followed curved pathways. The relationships between length of pathway, direct distance from source of vibrations, and speed of locomotion were analysed. Measurements of sound intensity in the experimental tank indicated that intensity gradients existed along the pathways followed by the fish. Further measurements of low frequency vibrations in the water are in progress.

PART I. REVIEW OF THE LITERATURE

The earliest notations on the ability of fish to hear were those of Aristotle and Pliny, who gave clear indications of their belief that fish could hear. Casserius in 1610 believed in the presence of internal ears in fish. In the eighteenth century the ears of fishes were studied by several workers, but not until the nineteenth century did scientific research in this field really begin to make progress.

Weber (1820) studied the bone connections which form a bridge between the swim bladder and labyrinth in certain fishes (Ostariophysi). He supposed these ossicles, later named after him, to be connected with hearing. In experiments with Ostariophysi and non-Ostariophysi he got postive results only with the former.

The general opinion among scholars of the last century was that the organs of the ear in fish were for hearing. The first noteworthy opposition to this opinion came from de Cyon in 1878 who observed the inability of lamprey eels to hear. Upon removal of the ears in one specimen, he noticed great disturbance in locomotion and established the purpose of the semicircular canals as organs of equilibration.

In 1884 Beard brought the two organ systems, those of the ear and lateral line, phylogenetically together by claiming the ear to be a modified portion of the system of the lateral line. Ayers further developed Beard's idea in 1892. The chief morphological facts upon which his view is based are the similarity of the

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[1]Received for publication April 10, 1953.

ear and lateral line in regard to ontogeny, structure of the adult organs and innervation.

Lee (1898) supports and furthers the work of Beard and Ayers and claims that among vertebrates, audition exists only when a group of sensory end-organs, called the *papilla acoustica basilaris*, is present. These organs are seen as an offshoot from the lagena in amphibians, and in higher vertebrates constitute the nervous portion of the organ of Corti of the cochlea. Lee asserts that the absence of the papilla acoustica basilaris, in fish, is a strong point against the possibility of hearing in this animal, and insists that the sole function of the ear in fish is equilibration.

Kreidl (1896) made experiments on the goldfish (*Carassius auratus*), in some of which he extirpated the ear; from these observations and others made on trout, he became convinced that hearing through the "auditory" organ in these fish was not possible. His observations led him to believe, however, that sound waves are perceived possibly through some specially developed skin sense. Kreidl's work was strongly supported by Lee who made similar observations from almost the same experiments.

Zenneck (1903) experimented on *Leuciscus rutilus, L. cephalus* and *Alburnus lucidus* in their natural environment. He observed clear responses resulting from the stimulus of tones produced under water.

Parker (1903) did not support the conclusions of Kreidl and Lee. He was interested in the possibility of sound vibrations stimulating not only the skin but also the organs of the lateral line and the ear. From experiments on *Fundulus* he concluded that fish definitely possessed the ability to hear.

Bigelow (1904) checked Kreidl's experiments by repeating the experiments of both Kreidl and Parker, using goldfish (*Carassius auratus*). His findings showed discrepancies in Kreidl's results, and also that Kreidl's operation left behind the very part of the ear most likely to be concerned with hearing. Bigelow indicates the sacculus-lagena as the organ concerned with sound perception.

Following these results there appeared a series of papers that were in part favourable to the opinion that fishes could hear and in part opposed to this view. Körner (1905) tested 25 different kinds of fish, mostly Cyprinidae. He concluded that fish do not hear.

Neither Brüning (1906), experimenting with stickleback, nor Marage (1906) obtained responses to sound.

Out of 23 species Maier (1909) obtained positive results only with *Ameiurus nebulosus, Siluris glanis* and the "American dwarf-fish".

Bernoulli (1910) repeated the experiments of Zenneck and tested the effect of various sound stimuli on *Salmo, Perca* and *Thymallus* in an open stream. From his observations Bernoulli concluded that fish do not hear, but do respond visually or tactually to water disturbances.

On the other hand Piper (1906) successfully identified an action current in auditory nerve preparations, resulting from sound stimuli in the water. He concluded that fishes respond to sounds by means of their ears.

Parker (1909), experimenting with the dogfish, *Mustelus canis*, was of the

opinion that this fish possesses ears capable of hearing which are also concerned with equilibrium and muscular tonus, and Meyer (1909) was successful in training fish to distinguish between two tones of different frequency.

Haempel (1911) experimented with *Ameiurus nebulosus, Gobio fluviatilis, Scardinius erythrophthalmus, Cyprinus carpio* and *Trutta fario*, in his studies on the responses of fish to underwater tones. He concluded that, in general, Salmonidae and Cyprinidae have the ability to respond to tones whereas Siluroids do not have that ability. He also showed that younger fish have a stronger ability to respond than older fish. Haempel recognizes both static and acoustic functions of the ear.

Körner (1916), working with *Ameiurus* and other species, got no results with tones from 659 to 3,520 cycles per second using sources in the water or outside of the aquarium.

Parker and Van Heusen (1917) presented experimental results showing that the ear of *Ameiurus nebulosus* is stimulated by slow vibratory movements of whole masses of water. They obtained positive reaction to tones of underwater source ranging from 43 to 688 c.p.s., but not to tones of 1,376 and 2,752 c.p.s. nor to the sound produced by a whistle blown outside the water. Water currents and surface vibrations were also without effect.

McDonald (1921) studied the ability of the bluntnose minnow (*Pimephales notatus*) to form associations with sound vibrations. She succeeded in training the fish to respond to a tone of 96 c.p.s. and concluded that the fish perceived the vibrations through their hearing sense.

Westerfield (1921) observed that mudminnows (*Umbra* species) could be trained to discriminate between two tones (288 and 426 c.p.s.), whereas von Frisch (1923) succeeded in training a blind *Ameiurus nebulosus* to form associations of sound vibrations with feeding.

Manning (1924) studied hearing in the goldfish (*Carassius auratus*) in relation to the structure of the ear. He observed the goldfish to respond to tones produced under water ranging from 43 to 2,752 c.p.s. and found the utriculus of the ear to be the receptor for vibrations from about 43 to 688 c.p.s. Above 688 c.p.s. the sacculo-lagena is the receptor, but Manning considers the lagena more important. The function of the sacculus in sound perception and the upper limit of perception for the goldfish were not determined. Manning also found no evidence of pitch discrimination in this fish.

Froloff (1925) experimented with *Perca fluviatilis, Acerina cernua, Tinca vulgaris, Carassius carassius, Crenilabrus griseus, Gadus morrhua, Gadus aegle-finus, Cottus scorpius* and *Corvina nigra*. He found that vibrations from sound sources in the water produced conditioned response faster than the vibrations from sources in air.

Bull (1928) succeeded in establishing conditioned responses in the wrasse (*Crenilabrus melops*) using a tone of 128 c.p.s. together with feeding. Similar results were obtained with the common eel (*Anguilla vulgaris*). Negative results were arrived at with *Blennius gattorugine*, whereas with *Gasterosteus aculeatus, Cottus bubalis, Pleuronectes platessa* and *Gabis minutus* results were indecisive.

Burlet (1929) worked with Physostomi and concluded that the sacculus is an organ of sound perception and has no relation to the sense of equilibrium.

Stetter (1929) found the upper limit of perception for blinded *Phoxinus laevis* (between 4,645 and 6,960 c.p.s.), *Idus melanotus* (5,524), *Carassius auratus* (3,480), *Cobitus barbatula* (1,740–3,480) and *Ameiurus nebulosus*, (13,139 c.p.s.). *Phoxinus laevis*, learned to distinguish two tones separated by a major third, in the range 821–651 c.p.s., and a minor third, in the range 290–345 c.p.s. Stetter found *Phoxinus* capable of remembering absolute tones after lapses in training from one to nine months. He found in some cases that up to five tones could be remembered at the same time and that the fish could select the feeding tone out of two or three different tones sounded simultaneously. In the non-Ostariophysi only a few species showed the ability to distinguish tones.

Stetter was the first to study sensitivity of hearing in fishes. By varying the intensity of his source outside the aquarium, he established relative threshold values for *Phoxinus laevis* and *Ameiurus nebulosus*.

Denker (1931), after an initial series of inconclusive experiments, finally found that *Phoxinus laevis, Idus melanotus,* perch and brook trout have sense of hearing. The labyrinth would pick up tones in the middle and upper parts of the tone scale.

Von Frisch and Stetter (1932) state the probability that hearing is present in fish in general, but that hearing sensitivity is greater in the Ostariophysi. They are convinced that the pars superior of the labyrinth of *Phoxinus laevis* has only an equilibrium function and plays no part in the perception of sound. The pars inferior is the hearing organ with a range of from 16 to 5,000–6,000 c.p.s., and plays no part in equilibration.

Moorehouse (1933) performed experiments on *Cymatogaster aggregatus, Oligocottus maculosus, Leptocottus armatus, Sebastodes caurinus, Platichthys stellatus, Lepidopsetta bilineata* and *Parophrys vetulus*. He tested the reactions of these fish to various noise-producing sources and succeeded in establishing conditioned responses in some species. He concludes that noise in general is not a great factor in disturbing fish in their natural surroundings, but admits that his experiments do not decide which senses are principally affected by noise vibration.

Von Boutteville (1935) repeated Stetter's experiments on hearing sensitivity using a tone of 652 c.p.s., and confirmed Stetter's results regarding the excellent hearing sensitivity of *Phoxinus laevis*. She experimented also with three characinids (*Hyphessobrycon flammeus, Hemigrammus caudovittatus* and *Pyrrhulina rachoviana*) and obtained definite reactions at an intensity of 21 phon, whereas negative results predominated at an intensity of 7–15 phon.[2] She considers 13 phon to be the approximate threshold. In *Gymnotus electricus* (Gymnotidae), positive results were not yet obtained at 35–40 phon. Stetter and von Boutteville found that *Phoxinus laevis* (Cyprinid), *Ameiurus nebulosus* (Siluroid), and three characinids had the same hearing sensitivity as man at 652 c.p.s. For *Phoxinus*

[2]The zero point of the German "phon" scale is equivalent to the threshold value of the human ear. One "phon" = one decibel.

laevis, von Boutteville repeated the threshold experiments at a frequency of 1,740 c.p.s. and obtained approximately the same results. The upper limit of sound perception for the three characinids was 6,960 c.p.s., and for *Gymnotus electricus* 870–1,035 c.p.s.

Von Frisch and Dijkgraaf (1935) found that *Phoxinus laevis,* in their natural habitat were unable to locate the source of sound. When strong sound sources created shaking in the water, an orientation occurred at a short distance from the source. This was attributed to the skin sense rather than to hearing. *Phoxinus* were able to locate sound sources if sufficiently strong. Von Frisch and Dijkgraaf believe that the fish were led by increasing intensity on approaching the source of sound. Whether skin or ear was responsible remained undecided.

Reinhardt (1935) observed that *Phoxinus laevis* and *Ameiurus nebulosus* were unable to determine the direction of a source of noise (buzzer) or of a tone at 20.6 c.p.s. In some cases a positive perception of direction occurred when the fish was within a proximity of 10 to 20 centimeters of the source. By applying a stimulus with simultaneous feeding, Reinhardt succeeded in producing an orientation reflex which continued after elimination of lateral line organs, ear and Weber's ossicles. He attributed the reaction to the function of the skin sense.

Tomaschek (1936) supports the theory that the lagena is the organ of hearing in fish and that the sacculus plays no part in this function.

Farkas (1936), starting as early as 1931, experimented with *Ameiurus nebulosus, Misgurnus fossilis* (Ostariophysi), *Upomotis aureus, Rhodeus amarus* (Physoclistae) and especially *Lebistes reticulatis* (Physoclistae). In all of these fishes Farkas was able to show the ability of hearing. With *Siluris glanis* (Ostariophysi) results were negative. In *Lebistes,* a fish without Weber's ossicles, the optimum reception determined lies between 86 and 435 c.p.s. Farkas found that higher tones (640 c.p.s.) were perceived only by younger fish (8–12 months) and not by older animals (over two years) except at a higher intensity. Upper limits of perception were also determined for *Lebistes* (between 1,200 and 2,068 c.p.s.), *Ameiurus* and *Rhodeus* (between 2,093 and 4,186 c.p.s.). *Lebistes* reacted to tones as low as 44 c.p.s. *Rhodeus* responded to tones of low intensity to which *Lebistes* did not react. Farkas attributes this difference in sensitivity to the presence of Weber's ossicles in *Rhodeus. Lebistes* possessed a definite timing sense in relation to their feeding period and were capable of identifying a feeding tone produced together with intense noise. Reactions of fish to tones started at the age of six to seven months and the ability of sound perception seemed to increase in the following generations. Farkas discovered that sound perception differs widely among individual fish even in the same species. He concluded that sound waves can stimulate a labyrinth without Weber's ossicles. This author gives a detailed morphological account of the acoustic cristae in *Lebistes,* outlining the differentiated parts of the cupula and ampullar apparatus and the effect produced in them as a result of stimuli.

Von Frisch (1936) presented an extensive review of the work of Stetter, von Boutteville and von Frisch. According to von Frisch reliable reactions to sound stimuli were proven in 32 species of fish (14 families) and were obtained by training methods.

Von Frisch (1938) studied the rôle of the sacculus and lagena for hearing in fish. Working with *Phoxinus laevis*, he found that the otolith in the sacculus as well as in the lagena regenerated if the sense epithelium was preserved after removal of the otolith. Even without the otolith the sense epithelium was capable of perceiving tones. The sacculus alone was found to perceive tones in the whole hearing range of *Phoxinus* (24.5–3,520 c.p.s.). Von Frisch thought that the lagena alone would perceive tones from 98 to 3,520 c.p.s. Without the pars inferior, *Idus melanotus* reacted to tones from 349 to 659 c.p.s., whereas *Phoxinus* did not. As in *Phoxinus*, the sacculus of *Idus* perceived tones of the whole hearing range (130–5,274 c.p.s.), whereas the lagena in this fish could perceive a slightly wider range than in *Phoxinus* (261–563 c.p.s.). Von Frisch emphasized the special perception mechanism of the Ostariophysi, the families of which include the Cyprinidae, Siluridae, Charicinidae and Gymnotidae. He described how the sound waves are conducted through the swimbladder and Weber's ossicles to the saccular otolith in *Phoxinus*, whereas the lagena seems to receive sound stimuli directly through the lagena "window" in the lateral wall of the skull. He mentioned that in the non-Ostariophysi, which are less sensitive to sound stimuli, there may be other structures not yet investigated, serving to increase the sensitiveness of hearing. Von Frisch found the upper limit of perception of *Phoxinus laevis* to be between 5,000 and 7,000 c.p.s., and that of *Ameiurus nebulosus*, above 13,000 c.p.s. For *Phoxinus*, he concluded that sounds from 25 to 130 c.p.s. were perceived by both the labyrinth and the skin sense organs, whereas tones from 130 c.p.s. up were perceived by the labyrinth alone.

Diesselhorst (1938), experimenting with non-Ostariophysi, observed that *Anguilla vulgaris, Marcusenius isodori, Lebistes reticulatis, Anabas scandens, Periophthalmus koelreuteri, Umbra krämeri* and *Lepomis* species perceived sounds. No reaction to sound could be obtained with *Perca fluviatilis* and *Pantodon bucholtzi*. He attributed the negative results to the experimental methods. Diesselhorst observed that *Marcusenius isodori* (Mormyridae) and *Anabas scandens* (Labyrinthici) have labyrinth spaces holding gas or air, and that these fish show an especially good ability to hear. The upper limit for sound perception in the eel was found to be between 488 and 651 c.p.s., whereas the lower limit was 36 c.p.s. The upper limit in *Marcusenius* was between 2,069 and 3,100 c.p.s., in *Anabas* over 659 c.p.s. and in *Lebistes* between the double-barred octaves. The Mormyridae could distinguish a tone difference of one-quarter octave, whereas the eel was unable to distinguish less than one whole octave.

Farkas (1938) investigated the sound-conducting apparatus in teleosts (*Lebistes*). He gives a detailed morphological description of the labyrinth and indicates specific parts which may function as tone-conducting pathways and effect a good hearing potentiality in many Ostariophysi and non-Ostariophysi.

Stipětic (1939) studied the hearing organ of *Gnathonaemus* (Mormyrid) of which he gave a detailed anatomical description. The upper limit of tone perception in this fish was between 2,794 and 3,136 c.p.s. Intervals of one tone could be distinguished by the fish. Up to this time such fine distinction had been demonstrated only for certain minnows.

Wohlfahrt (1939) extensively studied the ability of blinded *Phoxinus laevis*

to distinguish tones. The fish were able to distinguish between a continuous tone and an alternating sequence of the same tone with another one. Wohlfahrt found, however, that the signals were being distinguished because of their rhythmical interruption. Further experiments showed that the fish were able to distinguish between one and two impulses of the same tone per unit of time. When both feeding and warning signals were produced alternately in short staccatos, no clear distinction could be obtained. *Phoxinus* were able to distinguish a difference of a half tone in the range of the double-barred octave. Wohlfahrt observed a much greater capacity for relative tone distinction in *Phoxinus* than did Stetter. Minnows distinguished a half tone (987.7–1,046.5 c.p.s.) which is approximately a 6 per cent frequency distinction. Since the fish could not recognize a quarter-tone difference, this author assumed that the threshold of differentiation for sound waves of this range (977 c.p.s.) lies approximately at a third tone (a 4 per cent frequency difference). Wohlfahrt could establish that the physiological tone scale of *Phoxinus* is at least one whole tone, in the range of 587–1,568 c.p.s. He is convinced that his fish distinguished the tones by their frequencies and not by intensities. *Phoxinus* could not be trained to differentiate varying intensity of the same tone by means of the method employed in these experiments.

Schneider (1941) investigated the importance of the air chamber which borders directly on the sacculus in the Mormyridae. The fish he tested include *Macropodus opercularis, M. cupanus, Trichogaster trichopterus, T. leeri, Betta splendens* and *Colisa lalia.* The intimate relationship of the air chamber with the labyrinth gives the fish outstanding advantages for hearing. The upper limit of hearing for these fish was between 2,637 and 4,699 c.p.s. With the air chamber removed it falls to 494–659. Normal *Macropodus* differentiated intervals of from one and one-third octaves up to a sextet. Mormyrids with the chambers disconnected also showed a marked reduction in sensitivity of hearing.

Williams (1941) refutes the statement of von Frisch, that reactions to unconditioned stimuli in fish are unreliable. He observed clear reactions in some unconditioned fish to external sound stimuli, although he was unsuccessful in similar trials with *Carassius auratus.*

Graham (1941), in a comparison of the hearing organs of fish and mammals, contrasts the simple hearing organ of the fish to the mammalian cochlea and expresses his feeling that fish must be hard of hearing except in the special cases where we know to the contrary. He also feels that the degree of hearing must vary from species to species since the acoustic region of the brain shows varying degrees of development, even within one family. In commenting on Williams's experiments with fish, Graham agrees that the fish heard the sound stimulus but reacted to it as to a disturbance of the normal environment to which the fish was conditioned.

Mislin and Manger (1944) studied the reactions of untrained fish to sound stimuli. The observer was equipped with a special diving helmet and placed himself in the tank with the fish. The fish, having become accustomed to the observer's presence, were seen to make short reactive movements in the direction of a sound source, strongly noticeable with high tones and weakly with low tones.

Lowenstein and Roberts (1948) made an oscillographic analysis of the gravity and vibration responses from the labyrinth of *Raja clavata* as well as from the utriculus of the pars superior. Equally good but qualitatively different gravity responses were recorded from the otoliths of the sacculus and lagena of the pars inferior. Response to stimulation of the elasmobranch labyrinth is not confined to the pars inferior. An upward extending portion of the utriculus, called the lacinia was also found to receive vibration impulses.

Dijkgraaf and Verheijen (1949) studied the ability of *Phoxinus laevis* to distinguish tones. With regard to some of Wohlfahrt's results, Dijkgraaf claims that memory rather than distinction of tones was involved. What Wohlfahrt claims to be a response to different frequencies may be a response to intensity. Dijkgraaf reminds us that Wohlfahrt did perform intensity experiments which were unsuccessful. Wohlfahrt therefore recognized frequency changes and not intensity changes as being effective in his experiments. Dijkgraaf first repeated Wohlfahrt's experiments. Blinded *Phoxinus* were trained on whole-tone intervals in different frequency ranges which were separated by one octave (400, 800, 1,600, 3,200 c.p.s.). He found that many of the fish were upset by the punishment given in response to the warning signal. This occurred especially in the higher range where they became confused and often mistook the warning signal for the food signal. Secondly, Dijkgraaf performed experiments without the use of punishment. He gave a rhythmically interrupted tone, designated as a neutral tone (corresponding to Wohlfahrt's feeding signal) for about ten minutes. Then without changing the sequence, the frequency of the tone signal was changed into an alternating pulsation of two tones. Every other pulse was at the frequency of the neutral tone. This signal constitutes Dijkgraaf's feeding signal which corresponds to Wohlfahrt's warning signal. Whereas Wohlfahrt observed that *Phoxinus* distinguished a half tone in the range of 400 to 800 c.p.s., Dijkgraaf found an interval as low as one quarter tone. Using Wohlfahrt's methods, Dijkgraaf was unable to get any tone distinction in the range of 1,600 to 3,200 c.p.s., but did in using the signal system described above. He showed that the distinction in this range was due to an intensity factor. Dijkgraaf therefore demonstrated the ability of *Phoxinus laevis* to distinguish tone intensity in the range of 400 to 1,600 c.p.s., and hence proved void the negative results of Wohlfahrt. The upper limit of frequency distinction was established at 1,260 c.p.s. In the absence of Weber's ossicles (including the sacculus), frequency distinction is maintained down to an interval of one-third, and therefore the lagena is able to distinguish frequencies almost as well as the whole pars inferior.

Dijkgraaf (1950) investigated the function of the ear labyrinth in marine fishes. He performed a series of experiments with *Gobius paganellus*, in which various parts of the pars superior and pars inferior were removed, in order to check the function of each. Marked disturbances of equilibrium occurred only with elimination of the pars superior. Hearing was affected only where the pars inferior was eliminated on both sides. The upper limit of hearing in *Gobius paganellus* (non-Ostariophysi) was between 600 and 800 c.p.s. After bilateral removal of the pars inferior, the upper limit dropped to 500 c.p.s. Clear

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reactions were obtained at 100 c.p.s. after elimination of the pars superior and organs of the laterial line. This response Dijkgraaf attributes to cutaneous tactile perception.

Griffin (1950) repeated some of the experiments of von Frisch (1936); von Frisch and Stetter (1935); Stetter (1929); von Boutteville (1935); and Diesselhorst (1938), and calculated through actual measurement that *Phoxinus* has a threshold of response to underwater sounds of about 21–35 decibels above 0.0002 dynes/cm². Similar calculations for the eel, a non-Ostariophysi, showed that this fish has a threshold of approximately 50–60 decibels above 0.0002 dynes/cm². Griffin points out, however, that

the estimates are based on many assumptions and approximations. The responses of the fish were not as precise and objective as rigorous experimental psychologists would desire; for the sound pressure had to be varied as much as 20 db above the level which yielded responses in 50% of the trials before 90–95% response was obtained. There is also the possibility that nodes of high pressure and complex standing waves within the aquarium produced local sound pressures (or even local water currents) considerably in excess of the values indicated by a hydrophone of ordinary size. In this case the fish might have given occasional responses when they swam through one of these nodes, but not have been able to perceive the average sound level which has been roughly estimated above. Finally it is not clear how closely we were able to duplicate the exact conditions of the earlier experiments with respect to acousticoupling between air and water. Accurate information concerning the thresholds of hearing in fish can be obtained only by experiments designed especially for that purpose, with adequate control of the acoustical situation and preferably with the fish trained to give clear and unequivocal responses to indicate whether or not they can hear a sound stimulus.

This author, studying the significance of sound production for orientation in fish, found that the sea robin (*Prionotus evolans*) perceived sounds of 100 c.p.s. at about 91 decibels above 0.0002 dynes/cm².

Autrum and Poggendorf (1951) reported on absolute threshold of hearing in *Ameiurus nebulosus*. Dijkgraaf (1952) studied hearing in the marine fishes *Gobius niger, Corvina nigra* and *Sargus annularis*. The upper limits of perception were respectively 800, 1,000 and 1,250 c.p.s. *Sargus* had the highest sensitivity, which was explained by the presence of extensions of the swimbladder in the immediate vicinity of the labyrinth. The limit of frequency distinction in these species varied from three-quarters to one and one-quarter tone (9–15 per cent frequency difference). Removal of the pars inferior on both sides affected but little the perception of tones between 100 and 200 c.p.s. but that of higher tones suffered. Perception for tones higher than 400 c.p.s. was entirely lost. Similar experiments with removal of the labyrinth indicate that the pars superior takes part in the perception of tones in these fish, but a final conclusion was not reached.

SUMMARY OF PUBLISHED WORK

In the following abstract an attempt is made to summarize the most important statements found in the literature on hearing in fish between the years 1921 and 1952. The authors do not wish to imply that the statements selected are the final and correct answers to the problems in question. It should be

realized that a great variety of sound-producing and receiving apparatus, amplifiers and other accessory equipment has been used by the various authors. Generally, no due consideration has been given by the authors quoted to the physical characteristics of vibrations in water and/or to general physical aspects applicable to these problems.

1. *The ability to hear*

This has been established for all species studied by means of modern experimental methods.

2. *The range of tone perception*

This varies with the species and lies in the range from 16 to over 13,000 c.p.s. A summary of the statements made in some of the more recent papers (since 1929) is presented in Table I, page 140.

3. *Distinction of tones of different frequency*

Statements about the ability of fish to distinguish between tones of different frequency are summarized in Table II, page 141.

4. *Organs involved in hearing*

The pars inferior (sacculus and lagena) of the ear is responsible for hearing, whereas the pars superior (utriculus and semicircular canal system) is concerned with equilibration. The pars superior may take part in the perception of tones in some species (Dijkgraaf, 1952). In the Mormyridae and Labyrinthici, range and acuity are increased by the connection of a gas chamber with the labyrinth. In the Ostariophysi, the connection of air bladder with ear by Weber's ossicles increases the ability of fish to hear.

The perception of very low frequencies is generally attributed to sensory organs in the skin. There is no proof that the lateral line sense organs play any part in the perception of sound vibrations.

5. *Sensitivity to absolute and relative tone intensities*

The only absolute values encountered in the literature are 21 to 91 decibels above 0.0002 dynes/cm^2 (Griffin 1950). Relative values referring to the human threshold for a given frequency vary widely.

The ability to distinguish between tones of different intensity in the range of 400 to 1,600 c.p.s. was stated by Dijkgraaf and Verheijen (1949).

6. *Localization of source*

Two of the three papers dealing with the ability of fish to localize the source of sound vibration present negative results (Reinhardt, 1935; von Frisch and Dijkgraaf, 1935). These authors state that only very strong sound stimuli enable the fish in the immediate vicinity of the source to localize it by "trial and error". The skin sense organs were believed to perceive movements of the water produced by very strong vibrations.

A third paper (Mislin and Manger, 1944) mentions that fish made directional movements toward sound sources.

TABLE I.   Range of tone perception in fishes, in cycles per second.

Family	Species	Frequency range (c.p.s.) Low	High	Author
Non-Ostariophysi				
CYPRINODONTIDAE	*Lebistes reticulatus*	44	1,200–2,068	Farkas (1936)
GOBIIDAE	*Periophthalmus koelreuteri*	..	up to 651	Diesselhorst (1938)
	Gobius paganellus	..	600–800	Dijkgraaf (1950)
	Gobius niger	..	800	Dijkgraaf (1952)
ANGUILLIDAE	*Anguilla vulgaris*	36	488–650	Diesselhorst (1938)
SCIAENIDAE	*Corvina nigra*	..	1,024	Dijkgraaf (1950)
	Corvina nigra	..	1,000	Dijkgraaf (1952)
SPARIDAE	*Sargus annularis*	..	1,250	Dijkgraaf (1952)
MORMYRIDAE	*Marcusenius isodori*	..	2,069–3,100	Diesselhorst (1938)
	Gnathonaemus sp.	..	2,794–3,136	Stipetic (1939)
ANABANTIDAE	*Macropodus opercularis* *M. cupanus* *Trichogaster trichopterus* *T. leeri* *Betta splendens* *Colisa lalia*	..	2,637–4,699	Schneider (1941)
	Anabas scandens	..	over 659	Diesselhorst (1938)
Ostariophysi				
CYPRINIDAE	*Phoxinus laevis*	16	5,000–6,000	von Frisch and Stetter (1932)
	Phoxinus laevis	25	5,000–7,000	von Frisch (1938)
	Idus melanotus	..	5,524	Stetter (1929)
	Carassius auratus	..	3,480	Stetter (1929)
CHARACINIDAE	*Hemigrammus caudovittatus* *Pyrrhulina rachoviana* *Hyphessobrycon flammeus*	..	6,960	von Boutteville (1935)
SILURIDAE	*Ameiurus nebulosus*	..	over 1,300	von Frisch (1938)
GYMNOTIDAE	*Gymnotus electricus*	..	870–1,035	von Boutteville (1935)
COBITIDAE	*Cobitus barbatula*	..	1,740–3,480	Stetter (1929)

PART II. OBSERVATIONS ON *SEMOTILUS ATROMACULATUS ATROMACULATUS* (MITCHILL)

This part deals with certain aspects of the perception of low-frequency vibrations in the cyprinid, *Semotilus a. atromaculatus* (Mitchill) the creek chub, with special emphasis on its ability to localize the source of vibration. In the experimental procedures, attempts were made to avoid certain errors of a physical nature, evident in earlier work, and to measure the vibrations produced in the water, as to intensity, frequency and wave characteristics.

A large number of investigators have experimentally studied various aspects of the perception of vibrations by fish but the problems whether fish are able to locate the source of vibration in the water, and by which mechanism, have barely

TABLE II.   Range of tone distinction in fishes.

Species	Range of tone distinction, in cycles per second	Author
Non-Ostariophysi		
Umbra sp.	288 and 426	Westerfield (1921)
Anguilla vulgaris	Less than one octave[a]	Diesselhorst (1938)
Gobius niger	$\frac{3}{4}$ up to $1\frac{1}{4}$ tone[a]	
Corvina nigra	(9–15% frequency distinction)	Dijkgraaf (1952)
Sargus annularis		
Marcusenius isodori (Mormyridae)	$\frac{1}{4}$ Octave[a]	Diesselhorst (1938)
Gnathonaemus sp. (Mormyridae)	One tone[a]	Stipetic (1939)
Macropodus opercularis (Mormyridae)	Varying between one tone and $1\frac{1}{3}$ octaves[a]	Schneider (1941)
Ostariophysi		
Phoxinus laevis	A major third in 821–651 range; a minor third in 290–345 range	Stetter (1929)
Phoxinus laevis	$\frac{1}{2}$ tone in 987.7–1,046.5 range (6% frequency distinction)	Wohlfahrt (1939)
Phoxinus laevis	$\frac{1}{4}$ tone in 400–800 range	Dijkgraaf and Verheijen (1949)

[a]The frequency range in which distinction of tones was examined was not mentioned by these authors.

been touched to date (Reinhardt, 1935; Mislin and Manger, 1944; Dijkgraaf and Verheijen, 1949). The structure of the ear in fish, as compared with that of the higher vertebrates, makes this a problem of importance (Reinhardt, 1935; von Frisch and Dijkgraaf, 1935). A sound approach to the study of these and related problems in the perception of vibration in fish is hampered by the scarcity of experimental knowledge on the physical aspects of transmission of low-frequency vibrations in water.

A major difficulty in experimentation in this field is the lack of speakers of sufficient output with straight-line response in the low-frequency range. Piezo electric crystals do have favourable response characteristics, but their output is inadequate for the required experimentation. These shortcomings of electro-magnetic and electrodynamic speakers have been overlooked by all but one author (Griffin, 1950), especially in studies on the ability of fish to distinguish tones of different frequency and intensity (Wohlfahrt, 1939; Dijkgraaf and Verheijen, 1949). The intensity of the sound produced by the speaker at different frequencies cannot be kept constant by simply maintaining a constant current in the speaker circuit. This would only be admissible if the speakers had a flat curve of response. The intensity of the vibrations produced by the speaker can only be established by actual measurement in the water. A new technical difficulty arises with such measurements, in that receivers are required with flat

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frequency response in the desired frequency range. Furthermore, the energy values involved in such experiments require amplification of high magnitude in order to be measured. Such amplification at low frequencies must be kept free of distortion and interference by extrinsic electromagnetic waves. Only specially constructed amplifiers of stabilized circuits containing highly selective band filters can satisfy these requirements.

Methods and Materials

Sources of Vibration

In our experiments two sources of vibration were employed. For vibrations above 20 c.p.s. an audio-oscillator was used, with a continuously adjustable frequency range. The output of the instrument could be regulated by volume control. A satisfactory sine wave was produced which was checked on a cathode ray oscilloscope.

Frequencies below 20 c.p.s. were generated by means of the apparatus shown in Figure 1. The hermetically closed metallic box A was covered by a rubber membrane B (diameter 1¾ inch) and connected by means of tygon tubing (5/16 inch bore) to rubber cups C, C^1 and C^2, which are covered with thin plastic membranes (diameter 1 inch). These cups were placed in the experimental aquaria and functioned as speakers. A T-tube with funnel D was inserted in the system, which was completely filled with water. Glass cocks made it possible to interrupt the vibrations leading from chamber A to one or more of the cups C, C^1, C^2. Membrane B was connected by means of a lever to the vertical rod E of an old-fashioned sewing machine from which the needle holder was removed. The excursion made by the membrane could be adjusted by changing the length of the lever arms. The flywheel of the sewing machine was replaced by a step-down pulley which was driven by an electric motor F through a reduction gear. Further adjustments could be made by means of a rheostat. Care was taken to eliminate all air pockets from the system in order to avoid noises produced by the compression and expansion of the air.

Speakers

Electromagnetic speakers were used as transmitters for vibrations above 20 c.p.s. They were made waterproof by being wrapped in thin plastic sheets and held in position in the experimental tanks by means of bars and adjustable
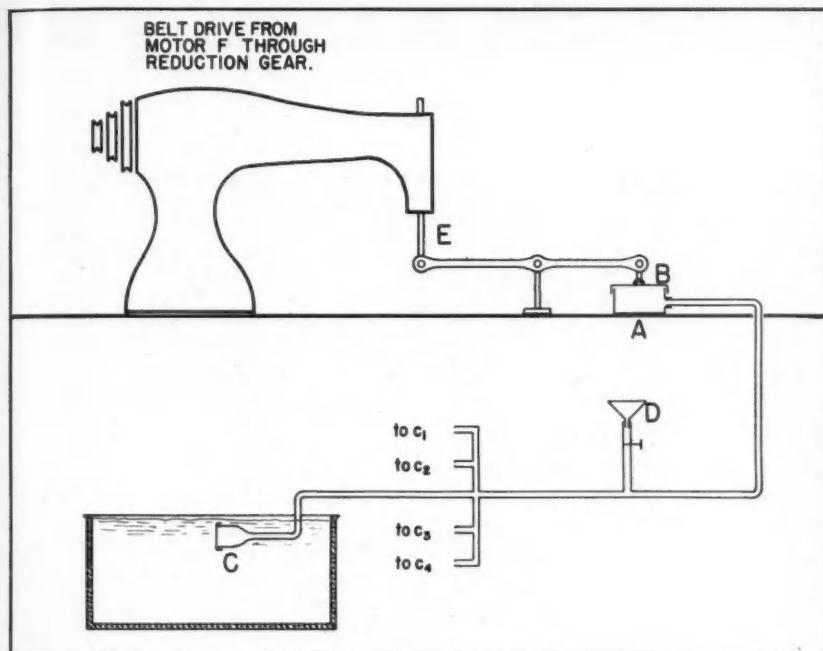
FIGURE 1. Low-frequency vibration apparatus.

goosenecks. The number of transmitters varied from one to four for each aquarium or tank. The speakers used for frequencies below 20 c.p.s. were described above.

AQUARIA AND TANKS

Experiments were carried out in aquaria (13 x 14 x 12 inches), cement tanks (47 x 42 x 35 inches) and in battery jars. Extraneous vibrations were reduced by placing the aquaria and jars on sheets of foam rubber. All aquaria were shielded in order to prevent the fish from seeing the movements of the experimenters. In some cases the fish could be observed by mirrors placed above the aquaria, whereas in other experiments observations were made by means of a movie camera.

RECORDING

One of the cement tanks had a false bottom of plate glass. The tank could thus be lighted from below, allowing for an analysis of the behaviour of the fish by means of motion pictures. A Paillard Bolex camera (Model H) with 15-mm. objective, was operated at a film speed of 24 frames per second by means of a Stevens electric motor with remote control. A Phipps and Bird timer was used to produce light flashes on the motion picture at intervals of one second. Lamps near the speakers were lighted simultaneously with the start of the

stimulus, thus indicating which of the speakers was in operation. All bulbs were placed so that the fish could not observe the light. A triple-pad volume control was connected with each of the four telephones to allow for finer adjustments of the output. Switches and volume controls were placed so that their operation could not be seen by the fish (Figure 2).
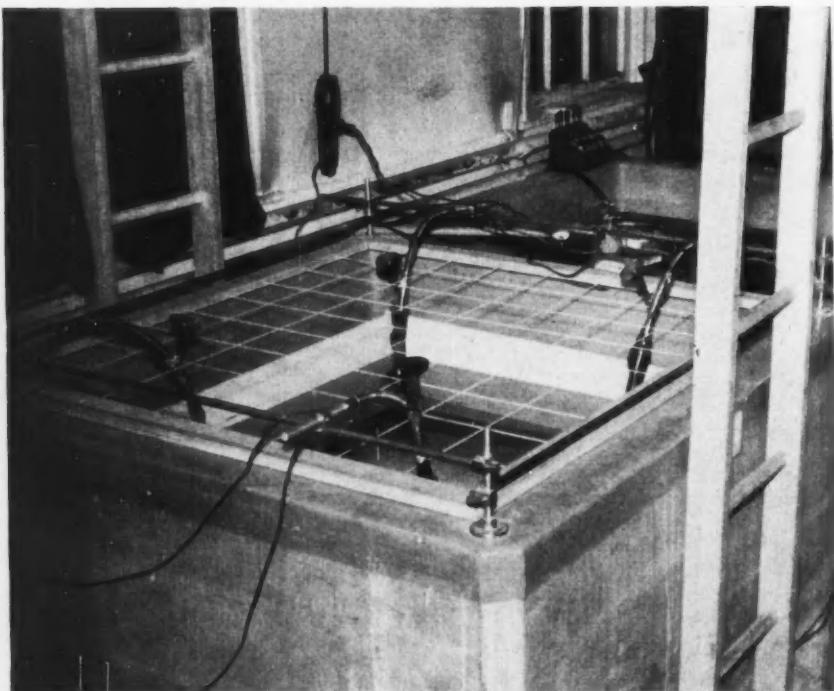

FIGURE 2. Experimental fish tank.

MEASUREMENT OF SOUND INTENSITY

The intensity of sound in the water was measured initially with a piezo-electric crystal of barium titanate (Brush Development Company), and two high-gain amplifiers (Hewlett Packard, Model 450A) in series with a Universal Avometer, Model 7. Some observations were made with an oscilloscope. The barium titanate crystal was attached to a vertical bar so that all comparative measurements could be carried out at exactly the same distance from the water surface and at accurately known positions in the tank. All the electrical equipment and the tanks were shielded with coaxial cables and fine-meshed copper screens connected to ground.

At the present time the absolute intensity of the vibrations in the water is being measured with two barium titanate crystals and a voltage standing wave

ratio meter. The characteristics of the waves produced by the speakers are studied with a wave analyser. The results of these measurements will be presented in a separate paper.

AUTOMATIC FEEDING DEVICES

Visual conditioning of the fish in the experiments was prevented by the use of mechanical feeding devices of remote control operated by means of solenoids.

EXPERIMENTAL PROCEDURE

Fish were trained to associate feeding with sound vibrations by submitting them to vibrations of a desired frequency, followed by the introduction of food near the source. In dealing with vibrations above 20 c.p.s. the fish were considered to be responding to a certain vibration only when they approached the speaker before being fed. At certain low frequencies (below 20 c.p.s.), excitement of the fish was considered a response.

Our experiments dealt with the study of: (1) Frequency range of perception of vibrations, (2) Distinction of tones, (3) Threshold of vibration perception, and (4) Movements of the fish in response to vibration stimuli.

1. FREQUENCY RANGE OF PERCEPTION OF VIBRATIONS

Upper limit. Fish trained at 50 c.p.s. by means of an audio-oscillator and speakers were submitted to vibrations of increasing frequency in order to determine their upper limit of perception. Initially it was found that the fish were easily conditioned to the closing of a switch in the electric circuit, so that the responses did not refer to the vibrations produced by the audio-oscillator. Subsequent changes in the circuit eliminated the interference by the switch.

Lower limit. The fish were trained at 12 c.p.s. by means of the apparatus described on pages 142–3 (Figure 1). The frequency of the vibrations was gradually decreased to establish the lower limit of perception.

Parallel experiments were carried out with fish in which the ears and/or the lateralis branch of the vagus had been removed.

2. DISTINCTION OF TONES

(a) *Ability to detect a change of frequency in a continuously sounding source.* Fish were subjected to a stimulus of 100 c.p.s. continuously applied for one hour without being fed. This stimulus will be further referred to as a "neutral tone". The frequency was then gradually lowered to 50 c.p.s. A sudden drop in frequency was avoided by allowing 8 to 10 seconds for the decrease. The fish were fed only at the lower frequency. After the fish had been trained to distinguish between the two tones, the frequency interval was reduced. This was done by gradually decreasing the frequency of the "neutral tone". In order to limit the conditioning of the fish to changes in frequency, the intensity was kept constant by means of volume controls. Experiments were also performed to ascertain whether fish responded to a change in intensity rather than to a change in frequency.

(*b*) *Absolute pitch.* "Neutral tones" of 100 c.p.s. were applied at intervals. Once or twice a day this tone was substituted by a tone of 50 c.p.s. followed by feeding. After the fish had learned to distinguish the "feeding tone", the frequency of the "neutral tone" was lowered in successive experiments until the fish ceased to distinguish between the two tones.

3. THRESHOLD VALUE OF VIBRATION PERCEPTION

Fish were trained to respond to vibrations of various frequencies above 20 c.p.s. For each of these the threshold value was determined. Intensities were measured by means of the apparatus described on page 144.

4. MOVEMENTS OF THE FISH IN RESPONSE TO VIBRATION STIMULI

Stimuli from one to four sources were given separately or simultaneously and at the same or varying relative intensities in the tank described on page 143. Motion pictures of the movements of the fish were taken and analysed.

EXPERIMENTAL RESULTS

1. FREQUENCY RANGE OF PERCEPTION OF VIBRATIONS

Upper limits. At the maximum intensity available, the upper limit of perception of vibrations was found to be from 5,700 to 5,750 c.p.s. In fish without ears (pars inferior and pars superior removed), the range of perception is still obscure because of the almost continuous activity of the fish and their inability to make a direct approach to the 'phone. This is caused by permanent loss of equilibrium in most of the fish. However, in so far as could be ascertained under careful observation, these fish perceived vibrations of from 20 to 200 c.p.s. Fish without the lateralis nerve behaved as normal fish.

Lower limit. The fish responded to vibrations of 1 c.p.s., which was the lowest frequency produced. The animals had been trained not to respond to the noise produced by the low-frequency vibration machine. This was done by running the apparatus, at intervals, with the cock below funnel D (Figure 1) in open position.

2. DISTINCTION OF TONES

(*a*) *Ability to detect a change of frequency in a continuously sounding source.* The fish could distinguish one-fifth of an octave in the range of 50–60 c.p.s.

(*b*) *Absolute pitch.* Trained on 50 c.p.s., the fish were able to memorize a neutral tone of 70 c.p.s.

3. THRESHOLD VALUE OF VIBRATION PERCEPTION

A threshold curve (Figure 3) was plotted, based on the measurement of relative intensity of vibrations measured with a cathode oscilloscope. The highest sensitivity was found to be at about 280 c.p.s., whereas the lowest was found at 20 and above 2,000 c.p.s. Below 20 c.p.s. accurate measurements could not be made. Considering the highest sensitivity at 280 as unity, the relative sensitiveness at 20 and 2,000 is .007 and .05.
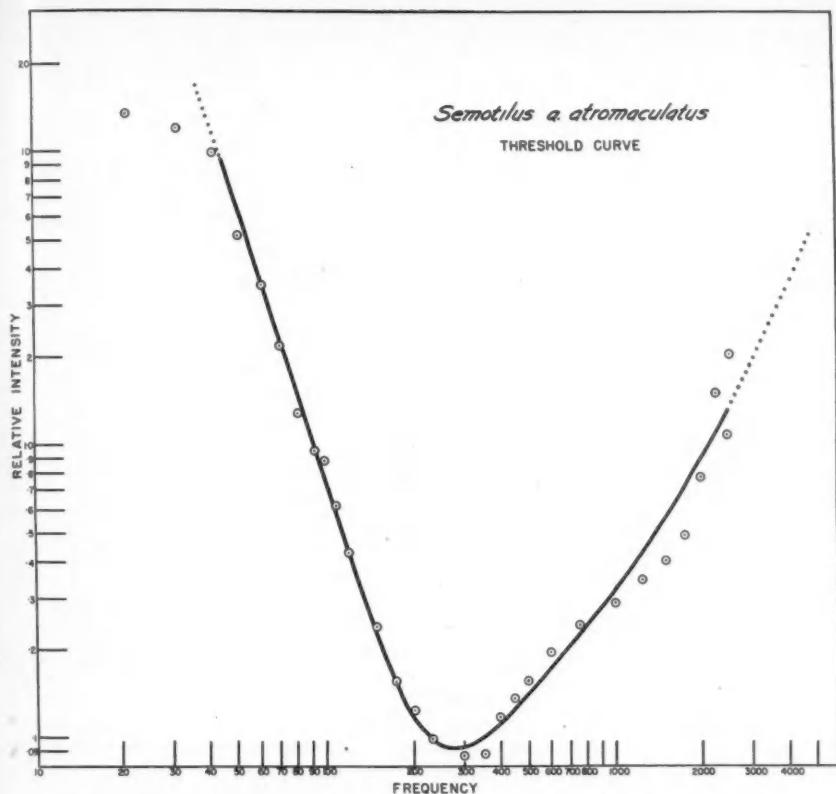
Semotilus a. atromaculatus

THRESHOLD CURVE

FREQUENCY

RELATIVE INTENSITY

FIGURE 3. Threshold curve of vibration perception in *Semotilus a. atromaculatus*.

4. MOVEMENTS OF THE FISH IN RESPONSE TO VIBRATION STIMULI

The analysis of the motion pictures, taken of the movements of the fish in response to vibrations of one or more sources sounded simultaneously at the same or different intensities, shows the following results:

(*a*) The fish are able to locate the source of vibration. This localization does not occur haphazardly. The fish move towards the source of vibration along curved pathways. Only after a number of fish have gathered around the source do other fish swim directly toward it. The shapes of the pathways suggest that the fish may be orientated by fields of higher intensity such as are produced by the crests of interfering waves, or by a standing wave, in the tank. This hypothesis seems to be strongly supported by analysis of Figure 4, in which the movements were traced of fish responding to the vibrations of one source. It can be seen from this tracing that fish which were near the upper central part of the figure at the start of the stimulus (encircled S in the tracing) did not move immediately toward any of the speakers, vibrating or not, but swam around
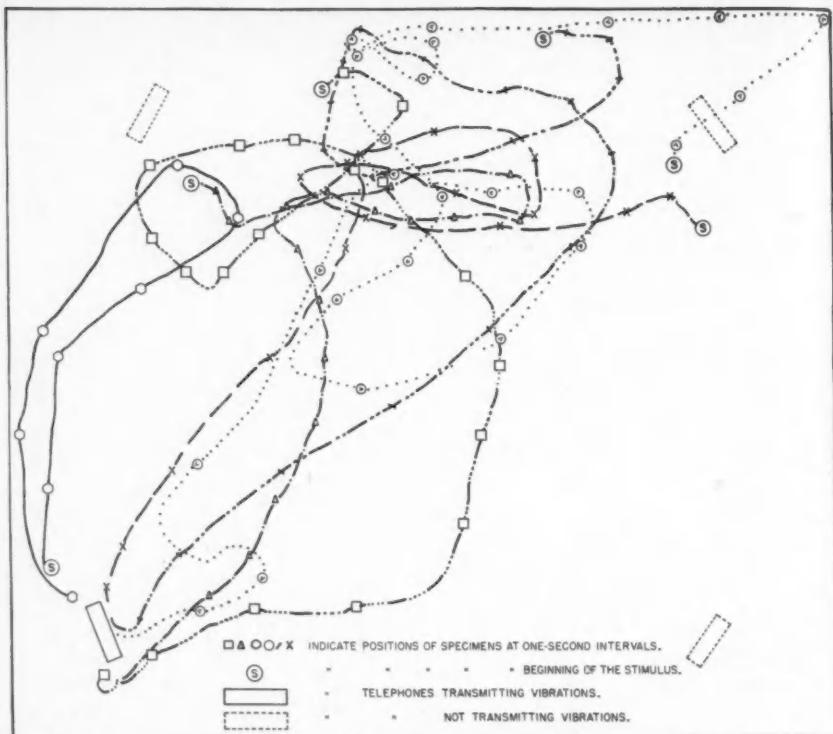
FIGURE 4. Pathways of fish in response to vibrations from one source.

at increased speed in the well defined region shown. Of the five fish present in this region at the start of the stimulus, four remained and swam around for 11–13 seconds after the start of the stimulus. The fifth fish remained as long as 18 seconds in the same area.

(b) Both direct observation and analysis of the motion pictures of the movement of fish in response to two sources of vibration of different intensity strongly suggest that, in locating the source, the fish are guided by fields of strongest intensity. Almost invariably the fish will move without hesitation towards the source of strongest intensity (Figure 5, left lower corner). It is remarkable that even fish which at the start of the stimulus were equally close to either source, did move toward the strongest source. It is to be noticed that in these experiments, the intensity of the weakest speaker was considerably above the threshold value for that frequency (50 c.p.s.) which was established by direct experiment. A problem of particular interest is that of the mechanism by which the fish are able to locate the source of vibration. If the gradient of intensity in the water is the guiding factor, the existence of such a gradient would have to be shown under the conditions of the experiment. In our experiments, however, the hori-
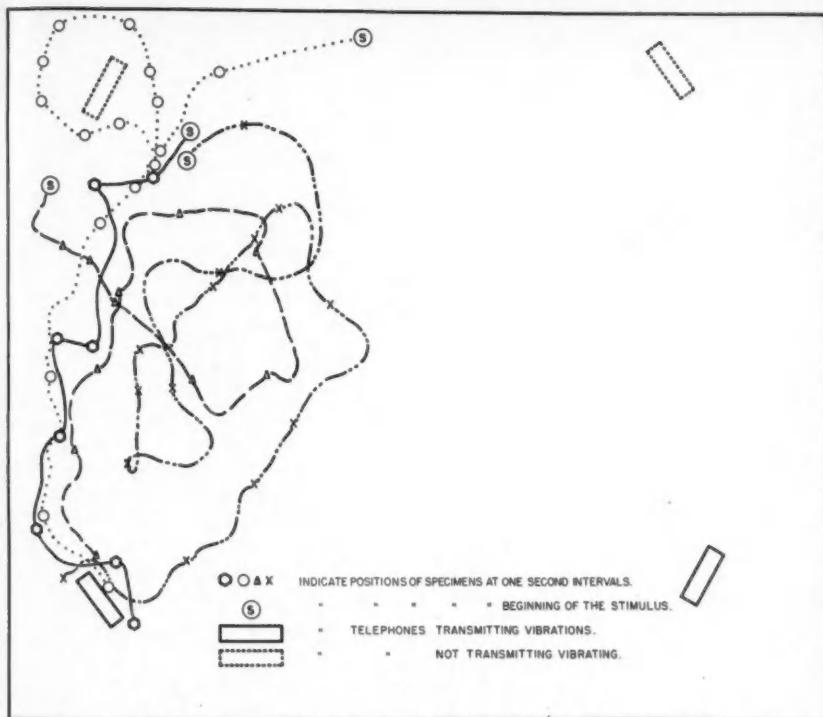
FIGURE 5. Pathways of fish in response to vibrations from two sources at different intensities but at the same frequency.

zontal dimensions of the tank were extremely small as related to the wave length of the vibration at 50 c.p.s. This means that the differences of intensity between the vibrations at the source and those arriving at the wall of the tank were minute. Nevertheless, the fish were able to locate the source with great ease, apparently following crests, which because of the small size of the tank and the resulting refraction must have followed an extremely complex pathway.

(c) Attempts to measure sound intensity in the tank with the use of a barium titanate crystal, and thereby establish a picture of the sound intensity gradient, showed that such a gradient exists along the pathways followed by the fish. Further measurements are being made by means of improved electronic apparatus.

(d) In Figure 6 the increment in speed per second interval is represented in graphical form, based on the analysis of a motion picture. Of nine fish, seven increased the speed of swimming in the first second interval after the start of the stimulus. Further analysis showed that the increment of speed is not related directly with the straight-line distance of the fish from the source of vibration.
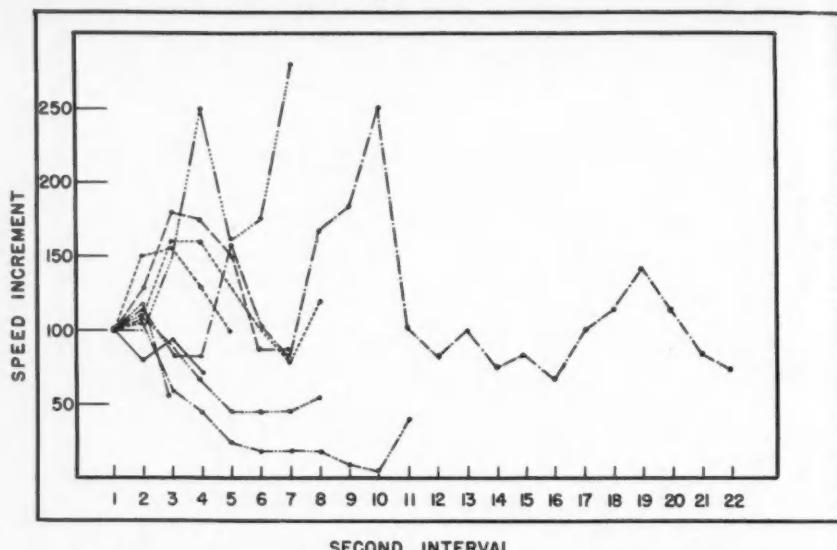
150

SECOND INTERVAL

FIGURE 6. Graph showing increment of speed of swimming in fish responding to a
vibration stimulus.

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Temperature Reception and Responses in Fish[1]

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CONTENTS

ABSTRACT

Conditioned-response experiments show that both bony fishes and selachians have surface thermal receptors. Electrophysiological studies have demonstrated in selachians two mechanisms which could provide continuous information about constant temperature conditions—the ampullae of Lorenzini and the lateral-line system. In other fishes only one such mechanism has been demonstrated, namely the trunk lateral-line system. Impulses from the ampullae and the lateral-line organs are, apparently, always being poured into the central nervous system at a rate which is characteristic of the temperature of the environment. The change in frequency of these action potentials with a given change in temperature is not great and there is no sign of adaptation. These sensory receptor mechanisms could operate in such a way as to give fish an absolute sense of temperature. In addition to this non-adaptive effect of temperature on these two kinds of receptors, there occurs, in the ampullae of Lorenzini only, another spectacular change in frequency of the nerve impulses with change in temperature, and this response is adaptive. This effect disappears with continued exposure to the new temperature, and the spontaneous impulses gradually assume the stable frequency which is characteristic of the temperature.

The principal effects of temperature on the activities of fish are as follows: Fish moving in a temperature gradient select a particular temperature because of an effect of the gradient temperatures on their movements. When fish move through the temperatures of a gradient, the frequency of their movements is least in the selected region. Moderately rapid changes of temperature do not elicit locomotor responses from resting fish until very high temperatures are reached, but do affect the frequency of movements of active fish. The frequency of spontaneous movements is related to the equilibration temperature, being greatest at the temperature ordinarily selected by the same fish if placed in a temperature gradient. Maximum cruising speed, as measured at different equilibration temperatures, is greatest at the selected temperature, as is also the distance moved in response to an electric shock. The maximum cruising speed that can be maintained by fish increases, with acclimation temperature, to a peak at the final preferendum.

Temperature selection by fish in a gradient is a function of surface thermal receptors

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[1]Received for publication on April 10, 1953; as revised, on October 26, 1953.

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not in the trunk lateral line, and of the forebrain. The relation between frequency of spon-
taneous movements and equilibration temperature depends in some way upon the integrity
of the cerebellum.

There are a few instances where a correlation has been demonstrated between temperature
conditions and behaviour of fish in nature because of the effect of temperature on activity.
There are other instances in which distribution of fish in nature appears to be correlated
with temperature as a result of selection responses to temperature gradients.

INTRODUCTION

IN THEORY there are two ways in which temperature can affect an organism. It
can have its effect by exciting thermal receptors, thus setting up nerve impulses
which go to the central nervous system. This may or may not lead to some overt
response on the part of the animal. Temperature can also affect an organism by
acting directly on the chemical processes in some or all of the tissues. Such an
effect, again, may or may not lead to some observable modification in the
behaviour of the animal. Undoubtedly, it is an action of the latter kind which
occurs in acclimation of cold-blooded animals to change in temperature level.
Knowledge of just what all the metabolic changes that constitute acclimation are
is incomplete. There is a certain amount of information on changes that occur
in oxygen consumption, opercular rhythms, enzyme systems and fats of fish with
prolonged exposure to a new ambient temperature (for references see Fox, 1939;
Fry, 1947; Freeman, 1950; and Christophersen and Precht, 1952), but it is
not known whether these changes are mediated by thermal receptors and the
central nervous system or whether they result entirely from the direct action
of temperature on all the cellular processes of the animal.

Several kinds of activity of fish have been observed and measured under
different temperature conditions. The present discussion, however, will be con-
cerned with only one form of activity—namely, locomotion; and the word activity,
when used with reference to the behaviour of fish, will refer to locomotion or
some aspect of it. The word "receptors" refers especially to cutaneous receptors.

EVIDENCE FOR CUTANEOUS THERMAL RECEPTORS IN FISH

Histological studies of fish skins have not revealed any specialized sense
organs except those of the lateral-line system. Herter could find no reference to
temperature sense organs in fish when, in 1925, he reviewed the available in-
formation on the temperature senses of animals. According to Kappers et al.
(1936) there are no complicated sensory terminations known in the skins of
vertebrates below reptiles and amphibians. Dijkgraaf (1940) states that as far
as he knows there is no information on temperature receptors in fish skin, and
Prosser et al (1950) say that cutaneous thermal receptors have not been localized
in fish.

Although thermal receptors, as histological entities, cannot be demonstrated,
their functional existence has been inferred because of results obtained by two
different experimental approaches.

The indirect approach, using the conditioned-response technique, has
furnished evidence indicating that fish possess a temperature receptor mechanism.

The first conclusive experiments providing this indirect type of evidence were those of Bull (1928, 1936–37). He subjected fish to a training program in which presentation of food was associated with an increase in environmental temperature. In these experiments the water, which flowed continuously through the fish tank, was warmed slightly by applying heat to the inflow tube for 15 to 60 seconds. Food was then introduced into the food chamber which the fish had to reach by swimming up a ramp. After a period of training the fish responded as soon as the slightly warmed water reached them, swimming into the food chamber even before the food was placed there. Of the several kinds of fish which Bull used, all came to react instantaneously to momentary increases in temperature of as little as 0.03° to 0.1° C. The time during which the fish were exposed to the slight temperature increase was so short and the response so prompt that there could be no doubt that reception by surface thermal sense organs occurred.

Dijkgraaf (1940) also employed the conditioned-response method. He too found that minnows and catfish could be conditioned to respond immediately to a momentary change in temperature of as little as 0.5°C. The alacrity of the response indicated that there was reception of thermal stimuli by surface receptors. There was insufficient time for internal receptors or chemical processes in the tissues of the fish to be affected.

Dijkgraaf was able to condition fish to respond to either an increase or a decrease in temperature, from which he concluded that fish, like other vertebrates, possess receptors for warmth and cold.

The direct experimental approach employs electrophysiological techniques. It is now generally agreed that the initial result of adequate stimulation of any sense organ is an immediate increase in the frequency of impulses in its sensory nerve. The magnitude of the change is dependent upon the intensity or magnitude of stimulation. If the stimulus continues at a constant level, impulses usually decrease in frequency and, in some types of sense organs at least, they cease entirely (Adrian, 1928; Hoagland, 1935; Fulton, 1946). Impulses in nerves from end-organs *known* to be thermal receptors have never been recorded in any animal, so it is not known whether receptors which respond to temperature change adapt completely to constant stimulation. It can be said only that in human subjects, from whom subjective information about sensation can be obtained, there is, apparently, some adaptation of thermal receptors, in that the intensity of warmth or cold sensations decreases with continued exposure to the stimulation temperature, but the sensations do not disappear entirely.

In experimental work with fish, it has been demonstrated that a change of temperature in the environment of certain sense organs causes a change in the frequency of impulses in the nerves supplying these organs.

Fuchs (1895) was probably the first investigator to attempt to record the effects of temperature on the impulses in sensory nerves of fish. He applied heat to the lateral-line organs of selachians, but did not observe any effect on the frequency of nerve impulses in the lateral-line nerves.

Modern amplifiers and recording devices were used in Hoagland's investi-

gations (1932–33, 1935) of lateral-line receptors. He discovered that the trunk lateral-line nerves in several species of fish are spontaneouly active, action potentials passing along them rhythmically, in the absence, as far as he was aware, of any mechanical stimuli. The frequency of these impulses varied with temperature, increasing with rising and decreasing with falling temperature. Over the range of temperatures studied (4–17° C.), the change in frequency for a 4° increase in temperature was slightly less than twofold. According to Hoagland's reports the receptors involved do not adapt to continued exposure to a given temperature. There is, in other words, a characteristic frequency for each temperature. Hoagland could get no impulses at all from spinal nerves or from the nerves supplying the head lateral-line organs, with changes in temperature.

These findings, together with Rubin's (1935) claim that fish without functional lateral-line nerves fail to make certain swimming movements, at a specific response temperature, which are characteristic of intact animals, led Hoagland (1932–33; 1935) to suggest that thermal reception in fish is a function of one of the types of end organs associated with the trunk lateral-line system.

Schriever (1934–35), also recording action potentials in the trunk lateral-line nerves of the cyprinid, *Barbus fluviatilus*, reported that changes of temperature had no observable effect on the spontaneous nerve impulses. This finding cannot be taken as contrary to Hoagland's results, however, for the nature of the preparations used in the two cases was quite different. Schriever's method appears to have been a rather gross one compared with Hoagland's and it seems likely that changes in frequency of nerve impulses with temperature would not have been observable in his records. Sand (1937) has directly confirmed Hoagland's observations.

Sand (1938) recorded the effects of temperature on the impulses in the sensory nerves of the ampulla of Lorenzini as well as of the trunk lateral lines of rays. The nerves from the ampullae are spontaneously active. The action potentials vary strikingly with changes in temperature. With a slight (0.4° C.) decrease in temperature in the vicinity of the end-organ the frequency of the impulses increases many fold. If the new temperature is maintained the frequency gradually falls until it becomes stable at a frequency below the former constant one. Similarly, with a slight increase in temperature, the frequency of action potentials immediately decreases spectacularly, but if the preparation is held at the new temperature the frequency gradually comes back to a constant level somewhat above the original one.

Sand also found, as Hoagland did, that spontaneous impulses continually pass along the trunk lateral-line nerves of rays. The frequency of these varies directly with temperature, but the magnitude of the variation is comparatively slight in response to relatively large changes in temperature.

Sand's and Hoagland's experiments provide direct evidence that receptor mechanisms sensitive to temperature are present in fish.

The conclusions to be drawn from these electrophysiological studies are strictly limited. The data demonstrate only that sense organs exist which meet

electrophysiological specifications for thermal reception in that there is conveyed from them to the central nervous system nerve impulses whose frequency is related to the temperature conditions of the environment. The data do not prove that these are the only thermal receptors or even that these receptors play any rôle at all in the responses of the animals to temperature.

It may be concluded from the experiments with conditioned responses that fish do have surface thermal receptors. Electrophysiological studies have demonstrated in selachians two mechanisms which could provide continuous information about constant temperature conditions—the ampullae of Lorenzini and the lateral-line system. In other fish only one such mechanism has been demonstrated, namely the trunk lateral-line system. Impulses from the ampullae and the lateral-line organs are, apparently, always being poured into the central nervous system at a rate which is characteristic of the temperature of the environment. The change in frequency of these action potentials with a given change in temperature is not great and there is no sign of adaptation. These sensory receptor mechanisms could operate in such a way as to give fish an absolute sense of temperature. In addition to this non-adaptive effect of temperature on the spontaneous impulses from the receptors of the ampullae of Lorenzini and the trunk lateral line there occurs, in the ampullae of Lorenzini only, another spectacular change in frequency of the nerve impulses with change in temperature, and this response is adaptive. This remarkable effect disappears with continued exposure to the new temperature, and the spontaneous impulses gradually assume the stable frequency which is characteristic of the temperature. In fish other than selachians no sensory receptors have been shown to be affected by temperature in this way.

THE RESPONSES OF FISH TO TEMPERATURE

The experiments quoted in the preceding section established the fact that fish do have surface thermal receptors capable of excitation by small temperature changes. They do not indicate whether stimulation of these receptors has any characteristic effects on the activity of fish. It is obvious that any modifications of activity that occur with alteration in temperature conditions need not *necessarily* be mediated through thermal receptors. It seems necessary therefore to consider first the effects of temperature on locomotion of fish without reference to thermal reception. In a later section the question of whether surface thermal receptors play a rôle in the production of these effects of temperature on activity will be taken up.

Experimental investigations of the effects of temperature on activity fall into three main divisions according to the temperature conditions employed. Thus, there are experiments in which an activity is observed as fish encounter or are subjected to rapidly changing temperatures; there are experiments in which an activity is observed at different temperatures after the fish have become equilibrated to them; and finally there are experiments in which fish are thoroughly acclimated to a given temperature before observations of an activity are made at that temperature.

First to be considered are the situations where rapidly changing temperatures are experienced. These conditions are encountered by fish moving in a temperature gradient and by animals placed in an environment where temperature, though uniform throughout, is changing rapidly.

Early observations of the behaviour of fish in temperature gradients, by Wells (1914), Shelford and Powers (1915) and by Cahn (1927) led these authors to the conclusion that the swimming movements of their experimental fish were affected instantaneously by small temperature differences encountered in the environment. However in all these experiments convection currents may well have played the rôle of responsible stimuli.

Doudoroff (1938) observed several kinds of marine fish in a more precisely controlled gradient than the ones used by previous workers. Upon introduction to the gradient the fish moved about but showed marked "avoidance reactions" to high and low temperatures of the range and spent the greater proportion of their time in one or two adjacent sections of intermediate temperatures.

Breder (1951), observing the movements of a school of fish in a pond, records the fact that the location and form of the whole school was controlled by the presence of certain "isothermal lines" in the environment. Small differences in temperature determined where the school would and would not go.

In our own experiments (Sullivan and Fisher, 1953 and unpublished) a horizontal temperature gradient has been used for observations of the behaviour of groups of young trout (*Salvelinus fontinalis*, 2–3 inches long). In this environment the trout move constantly to and fro, but spend more time in a particular, intermediate temperature range than in the other temperatures at their disposal. They may be said to "select" a certain temperature, and this behaviour shall subsequently be called "temperature selection".

It is, of course, obvious that fish which do not move in a gradient cannot be said to select a particular temperature from among those available. This fact is emphasized by Fisher and Elson (1950), who note that they considered it necessary to stimulate their fish to move in selection experiments.

Although most data are available for horizontal gradients, there are some for vertical temperature gradients. Graham (1949), for example, found that trout in a vertical gradient selected a particular temperature from among those available, and Brett (1952) has recorded the same behaviour in five species of Pacific salmon.

When selection of a particular gradient temperature is observed, it means that the net movement of animals is into this region. Fish must get to the selected temperature by a series of discrete movements. Movements do not cease when the selected region is reached; animals continue to move but come back to this region more often than they go to others. Some characteristic or characteristics of each movement must then be modified by the temperatures experienced as the fish move, in such a way that consecutive movements bring them to the selected temperature and cause them to spend more time there than elsewhere. Each movement can be completely described in terms of its length, its direction and the time it takes. Therefore, the response of moving fish to

stimulation by the temperatures of the gradient must result from modification of one or more of these components of movements by the different temperatures experienced (Fisher and Elson, 1950).

The relations of two of these components of movement to temperatures of a gradient have been investigated in our laboratory (Sullivan and Fisher, unpublished). The determination of frequency of movements per unit time is a convenient way of measuring the time of movements—that is, the average time elapsing between the beginning of one movement and the start of the next. When the frequency was studied in each of three sections of a temperature gradient it was found that trout make fewer movements *per unit time* in the section which includes the selected temperature than in the sections comprising temperatures higher and lower than that selected. The results represented the average frequency of movements made by trout as they swam many times back and forth through the whole range of gradient temperatures. Differences in frequency of movements in the different sections are small and could not, of themselves, account for temperature selection.

The relation of direction of movements to the temperatures of the gradient was also examined. Trout show no tendency to orient in any particular way to the temperature gradient, nor could any evidence be gathered to show that trout make more movements toward any particular temperature region than toward others.

No direct measurements have been made of the lengths of movements in a temperature gradient, but certain indirect evidence (Elson, 1942; Fisher and Elson, 1950; unpublished work of Fisher and Scott, Sullivan and Fisher) indicate that the length of individual movements may be modified as the fish move through different temperatures. Further experiments are necessary before a conclusive statement can be made.

It has been pointed out that fish must move when they select temperature and that their movements must be modified by the temperatures encountered. In this connection, however, it is not necessary that the movements be initiated or caused by the temperature conditions. They may be initiated by other stimuli, external or internal. The question whether locomotion is a response which *usually* follows as a result of changing temperature is not answered by these experiments in temperature gradients. There are data of a different kind that do relate to this question. They were obtained by observing fish initially at rest in an environment where temperature was changed rapidly.

Rubin (1935) placed fish in a container and heated the water at rates between 3° and 5° C./min. He did not begin an experiment until the animals were resting quietly on the bottom of the container. The fish remained inactive until a specific "response temperature" (approximately 27° C.) was reached, when they suddenly began vigorous swimming movements. Rubin does not mention what his animals did at temperatures above 27° C.

Dijkgraaf (1940) directed streams of water of different temperatures against the flanks of fish which were resting quietly on the bottom of an experimental tank. The stream flowed gently out against the skin of the fish and mixed with the tank

water so that the environmental temperature was not greatly altered by the modified water. The fish gave no response at all to water streams of temperatures up to 35° C. To temperatures above this they reacted by swimming away from the impinging stream of water.

Rubin's and Dijkgraaf's observations would indicate that temperature changes—at least in the lower part of the temperature range—which affect primarily the surface of the fish, do not ordinarily elicit from resting fish a response in the form of movement. It is only when the temperatures experienced approach the lethal level that movement seems to occur as a consequence of a sudden temperature change.

While movement is *initiated* by temperature change only in the higher ranges, the *modification* in frequency of movements of swimming fish by different gradient temperatures indicates that movements elicited by other means, including spontaneous movements, are affected by temperature change over a wide range. Wells (1914), for example, heated the water around fish at different rates, accomplishing the full rise in times ranging from five minutes to one hour. He observed an increase in activity with rising temperature. He wrote, ". . . at the beginning the fish in the experiment swam about exactly as did the one in the control. As the experimental temperature increased, however, the activity of the experimental fish increased likewise" (p. 3).

Well's observations are being repeated and extended in our laboratory. Results so far confirm Wells's impression of a change in frequency of movements with rapidly changing temperature. It appears that frequency of movements is, on the whole greater in the low- and high-temperature ranges than in the intermediate range which includes the selected temperature. Without doubt, therefore, the frequency of movements varies with temperature when conditions are such that the fish are moving and the change in temperature is rapid.

The responses of fish that have just been discussed deal with situations in which animals were subjected to relatively *rapid* changes of temperature either experimentally or because they moved through a gradient of temperature. The temperature changes were so rapid that it is most unlikely that the body temperatures of the fish were ever in equilibrium with the external temperatures. Next to be considered are effects of temperature seen when the organisms are permitted to come to equilibrium with the external temperature. Experiments have been done (Sullivan and Fisher, unpublished) in which temperature was changed to a new level and the animal allowed to equilibrate with it for a period of 20 minutes before measurements of frequency were made. This interval of time is long enough for the whole animal to come to the temperature of the environment (Gunn, 1942). Movements under these circumstances may be considered to be spontaneous, since there were, as far as the observer was aware, no changes in physical or chemical factors which might act to stimulate the experimental animals.

Frequency of movements under these conditions is least at low temperatures; it increases with temperature to a peak which occurs at the temperature normally selected by the same animals in a gradient; it then decreases as temperature is

raised further, reaching a second low at a temperature several degrees below the lethal level. With further increase in temperature, frequency increases again rapidly to a second peak, after which it decreases abruptly as the animal dies. This relation is the same whether temperatures are changed in regular sequence or at random.

It would seem from this result that for each temperature there is a general level of activity or frequency of spontaneous movements that is characteristic of it. The temperature level rather than temperature change would seem to be the significant factor determining activity under the conditions of these experiments. The rate at which the temperature changes thus appears to be very important, rapid rates of change producing a minimum frequency at intermediate temperatures, slow rates of change producing a maximum frequency at these temperatures.

The effects of temperature on other activities besides frequency of movements have been studied under conditions in which the fish were brought to equilibrium with an experimental temperature before observations were begun. Fry and Hart (1948) measured the maximum cruising speed that goldfish could maintain at each of a number of temperatures. They obtained values at each of a series of temperatures for fish from various acclimation temperatures. For goldfish from a given acclimation temperature, the maximum possible cruising speed increased with equilibration temperature to a peak at a temperature which corresponded more or less closely to the selected temperature of fish from the same acclimation temperature. Fry and Hart did not extend their observations to temperatures above that at which the peaks occurred.

Sullivan and Fisher have measured cruising speeds of trout at temperatures up to the lethal point. The results, given in Figure 1, show that after the first peak is reached, at a temperature corresponding to that selected in a gradient, the cruising speed falls off as temperature is raised further, but rises again just before the animals die. The similarity between the effects of temperature on cruising speed and on frequency of movements suggests that these activities are closely related.

Shaw, Escobar and Baldwin (1938) measured the average length of path for a given period of time (velocity) of goldfish swimming freely in a tank at each of several temperatures. Length of the path increased with temperature. No peak was recorded, but it is possible that in these experiments the temperature was not raised to the level at which such a peak might be expected.

The distance moved by trout and salmon fingerlings in response to an electric shock has been shown by Elson (1942) and by Fisher and Elson (1950) to be maximum at the temperature selected by the same fish in a gradient.

In these determinations of frequency, cruising speed and distance of movements, the fish were exposed for a short time to each experimental temperature before observations were begun. The time of exposure was sufficient for equilibration but not for acclimation to each temperature to occur.

The effects of change in acclimation temperature on temperature selected and on cruising speed of fish have been studied. The actual temperature selected
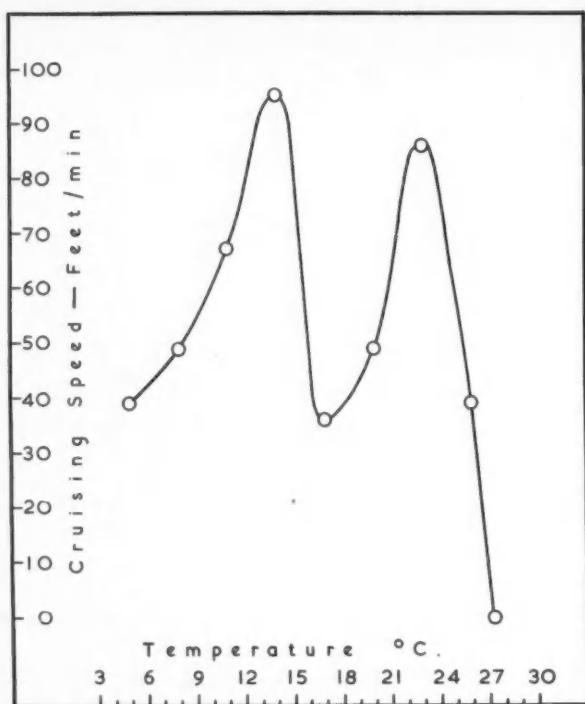
FIGURE 1. Maximum cruising speed of trout at various equilibration temperatures.

varies appreciably with the acclimation temperature in some instances (Doudoroff, 1938; Fry, 1947; Graham, 1949) but not in others (Brett, 1952). In general, when the selected temperature is affected by an alteration in the acclimation temperature, the change is in the same direction, but is not as great, as the change in acclimation temperature. The temperature at which the selected temperature and the acclimation temperature coincide, called the final pre-ferendum by Fry (1947), may be an ecologically useful characteristic.

Maximum cruising speed of which goldfish and trout are capable at each of a number of acclimation temperatures has been shown by Fry and Hart (1947) and by Graham (1949) to increase with acclimation temperature to a peak at a temperature corresponding approximately to the final preferendum temperature.

The principal effects of temperature on the activities of fish, discussed above, can be summarized as follows. Fish moving in a temperature gradient select a particular temperature because of an effect of the gradient temperatures on movements of the animals. When fish move through the temperatures of a gradient, frequency of their movements is least in the selected region. Moderately rapid changes of temperature do not elicit locomotor responses from resting fish until very high temperatures are reached, but do affect the frequency of

movements of active fish. The frequency of spontaneous movements is related to the equilibration temperature, being greatest at the temperature ordinarily selected by the same fish if placed in a temperature gradient. Maximum cruising speed, as measured at different equilibration temperatures, is greatest at the selected temperature, as is also the distance moved in response to an electric shock. The maximum cruising speed that can be maintained by fish increases, with acclimation temperature, to a peak at the final preferendum.

THE ROLE OF RECEPTOR–CENTRAL NERVOUS SYSTEM MECHANISMS IN TEMPERATURE RESPONSES

From the discussion in the last section it is evident that temperatures of a gradient and different constant temperatures have certain measurable effects on the activity of fish. The next question is, which, if any, of these effects depend on the operation of surface thermal receptors and the central nervous system.

It has been emphasized that selection of a particular temperature in a gradient occurs because of effects of the various temperatures, encountered as the fish move about, on the movements of the animals. The fish move through the gradient with such rapidity that it is improbable that their body temperatures could follow the external changes in temperature. The mechanism by which the movements are modified so as to bring about selection must therefore be put into operation by changes in temperature of the surface of the body only. It is impossible to imagine any means other than surface temperature receptors and the central nervous system by which such changes at the surface could affect the complicated and very well integrated series of muscle contractions which make up the normal swimming movements. Hence it seems necessary to conclude that a temperature sense plays an essential rôle in the selection of temperature. Direct evidence for this conclusion is provided by some recent experiments of Sullivan and Fisher (unpublished), in which the body surfaces of trout were desensitized by treatment with cocaine. Treated animals no longer responded to pin pricks or to the touch of a hot needle, and it was found that they did not select temperature in a gradient.

The possibility that the sense organs concerned in temperature selection might be a part of the trunk lateral-line system was experimentally investigated. Figure 2 illustrates temperature selection by a group of five trout before (Figure 2a) and after (Figure 2b) section of the trunk lateral-line nerves. The fish selected the same temperature range in both tests.[3]

It is apparent then that the sense organs of the trunk lateral-line system do not play an important part in the response of trout to a temperature gradient. Dijkgraaf (1940) also examined the possibility that thermal reception, in fish conditioned to respond to temperature changes, was a function of lateral-line

[3]It may be noted that in the second test, after section of the nerves, the spread of the distribution histogram, as measured by its standard deviation, was less ($\sigma = 3.60$) than it was in the first test ($\sigma = 3.87$). An increase in the precision of selection by a given group of fish characteristically occurs with repeated trials in the gradient up to the third or fourth trial (Sullivan and Fisher, in press).
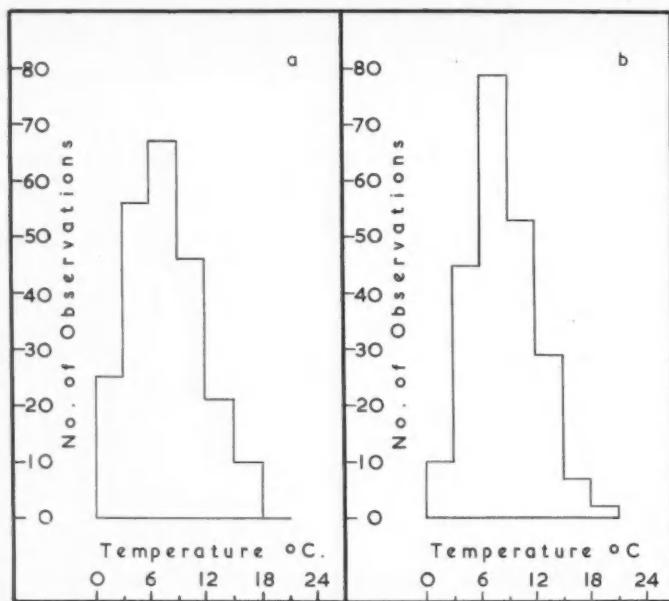
FIGURE 2. Temperature selection of trout. *a*. Intact animals. *b*. After section of the trunk lateral line nerves.

sense organs. He found that his conditioned fish responded to temperature stimuli as briskly as normal animals even though the whole lateral-line system was rendered non-functional by operative procedures. When, however, he cut the spinal cord and applied the stimulus behind the cut, the fish failed to respond, although they still reacted immediately when the thermal stimulus was applied to the body surface anterior to the cut. Dijkgraaf concluded that surface reception of thermal stimuli in his experiments was a function of cutaneous receptors scattered over the body surface. It seems likely that the conditioned response to temperature and the selection response to a temperature gradient depend on the same receptor-central nervous system mechanism.

Further evidence that a receptor-central nervous system mechanism is in operation in temperature selection is provided by the observation (Sullivan and Fisher, unpublished) that after destruction of the forebrain trout no longer select temperature in the way that normal fish do.

The relation of frequency of spontaneous movements to different equilibration temperatures has not been demonstrated to be dependent on surface receptors. It has been shown, in this laboratory, that the sense organs of the trunk lateral-line system are not essential in this response, but sectioning of the lateral-line nerves does render the response somewhat less precise. It has further been established that damage to the dorsal part of the cerebellum eliminates the peak in frequency which normally occurs at the selected temperature, so

that frequency increases regularly with temperature. Thus it would appear that there is, in the cerebellum, a centre whose integrity is essential in the control of frequency of movements at different temperatures. There is no proof yet, however, that the operation of surface thermal receptors is necessary to this response.

Present evidence indicates that the nervous mechanisms in the selection response to a gradient, and the frequency response to different equilibration temperatures, are quite separate. Damage to the dorsal part of the cerebellum does not affect the ability of trout to select temperature; destruction of the forebrain does not eliminate the frequency response to different equilibration temperatures.

Two main facts have now emerged, concerning the receptor-central nervous system mechanisms involved in the temperature responses discussed above. The first is that temperature selection by fish in a gradient is a function of surface thermal receptors not in the trunk lateral line, and the forebrain. The second is that the relation between frequency of spontaneous movements and equilibration temperature depends in some way upon the integrity of the cerebellum.

The question of participation of thermal receptors in the frequency response is still open. Under the conditions of the experiments there was ample time for the fish to come to equilibrium with each temperature before observations were made (Gunn, 1942), so that temperatures could have their effects by acting on chemical processes in the tissues generally, or in the central nervous system alone. If surface thermal receptors play a rôle in these responses they must be receptors which do not adapt to temperature changes very rapidly. Receptors of the trunk lateral line could conceivably operate here, since Hoagland has shown that they do not adapt to temperature. Action potentials from the trunk lateral-line nerves pass into the cerebellum (Hoagland 1934–35). When the lateral-line nerves are cut the cerebellum is silent. Hoagland has suggested that these impulses from the lateral line have a tonic reinforcing effect in the central nervous system. It may be that there is, in the cerebellum, a centre for control of frequency of movements in relation to temperature and that it is affected directly by temperature, while its function is reinforced by impulses from the lateral-line which also vary with temperature. Elimination of the lateral-line impulses, while not eliminating the frequency response, may make it less precise.

Acclimation temperature is known to affect temperature selection and cruising speed of fish. There is no experimental evidence whatsoever to suggest that thermal receptors and/or the central nervous system are important in determining the relation between acclimation temperature and these activities.

THE TIME OF DEVELOPMENT OF THE THERMAL SENSE IN FISH

Paton (1906–8) wrote "prompt and unmistakable responses to thermal stimuli appear at an early stage—for example energetic wrigglings occur in trout (*Salmo fontinalis*—14 mm.)—when touched with a warm needle, and the same is equally true of the selachians" (p. 543). These observations might signify

thermal reception, but they might just as well be evidence of tactile reception only.

Evropeyzeva (1944) presents evidence which indicates that thermal receptors are functional in turbot larvae on the first day after hatching. This is long before the yolk sac is absorbed. Yolk sac absorbtion is completed only after 11 days at a constant temperature of 8°C.

Experiments carried on in this laboratory have demonstrated that trout and salmon fry select temperature in a horizontal gradient at an age at which "the yolk sac has been practically absorbed" (Fisher and Elson, 1950), and indications are that the ability to select temperature is well established when the fry first become capable of sustained forward swimming.

THE ROLE OF RESPONSES TO TEMPERATURE IN MOVEMENTS AND DISTRIBUTION OF FISH IN NATURE

A great many authors have expressed the belief that temperature is an important factor in the control of movements and distribution of fish in nature. Wells (1913, 1914) gives references to some of the papers; Chidester (1924-25) reviews the opinions of a few early authors; Herter (1925) mentions others and Fry (1951) refers to some of the more recent papers. There can be no doubt that temperature does, in some cases, play a part in determining the movements and locations of fish.

In the preceding discussion, it was seen that a distinction exists between temperature acting as an influence on amount of activity and temperature acting as a determiner of where a moving fish will go. Thus, on the one hand activity may be initiated, increased or decreased according to the temperature of the environment. On the other hand, non-uniformities of temperature in the environment may modify movements and determine where the animals will go and where they will spend most time.

Either one or both of these responses may lead to a correlation between temperature and movements and distribution of fish in nature.

Foerster (1936–37) presents data that indicate that sockeye salmon begin to come to the surface of Cultus Lake when the average lake temperature rises to from 4.5° to 5.5° C.

White (1939) reports that the descent of salmon smolts from a brook in Nova Scotia was related to rise in temperature and low light intensity.

In an investigation of the responses of trout to current, Elson (1939) found that high water temperature (20° as against 17° C.) resulted in a decrease in or even a lack of swimming activity.

Dendy (1946) suggests that warming of the deep water of Norris reservoir causes bass to "become active" and move into the shallows.

In all of these cases the observations suggest that temperature has its influence either by initiating or by increasing or decreasing the activity of the fish, which then respond to a greater or less degree to other stimuli such as a temperature gradient, current, or light. These latter responses determine where the fish go.

In some field observations a correlation has been found between distribution of fish and temperature.

Jammes (1931) claimed that the vertical distribution of trout in Lake Oredon was determined by temperature. According to him, the fish stay in the 12° C. isotherm and move up and down with it.

In Foerster's observations (1937) the first indication of migration of sockeye salmon was that they began to appear in the surface waters in the spring. He suggests that the salmon, once they start to move in the spring, are "attracted to the surface strata by warming waters". He also presents some evidence to show that when surface water temperatures run higher than 13° C. migration ceases because the fish no longer come to the surface. The inference is that the salmon are brought to the surface by a response to a vertical temperature gradient, and when surface temperatures become very high their distribution is limited to deeper water by this same selection response.

Fry (1937) reported that cisco in Lake Nipissing migrate to deep water in the spring, apparently because of a selection response which takes them out of the warm surface layers and results in their spending the summer in the cool water below the thermocline.

Berzius (1949) found a correlation between temperature and distribution of fish in the Riga Sea. In this body of water thermal stratification is established early in the summer, but the position and thickness of the temperature layers are sometimes shifted by storms. He observed that the fish also shift their positions and are always to be caught in abundance in a particular temperature stratum.

Dendy (1945) found that the vertical distribution of fish in three storage reservoirs was correlated with temperature.

Baldwin's observations (1948), revealed that speckled trout in Redrock Lake occur at the depth where temperature is the same as the final preferendum temperature determined experimentally by Fry (1947) and Graham (1949), indicating that distribution is determined by a selection response to the vertical temperature gradient.

Hayes (1946) observed that the speckled trout in two lakes in Nova Scotia were found at a particular temperature rather than a particular depth.

It seems likely that in these cases the distribution of fish is the result of responses to the temperature gradients present in their environments. In order to so respond the fish have to move. It is not clear in any of the cases cited whether temperature conditions also provide the initiative to move, but in the case of Foerster's observations and Dendy's, the data, scant as they are, suggest that this may be the case.

Available data indicate, then, that there are instances when a correlation exists between temperature conditions and behaviour of fish in nature, because of the effect of temperature on activity. There are other instances in which distribution of fish in nature appears to be correlated with temperature as a result of selection responses to temperature gradients.

With a few exceptions, however, field data are not adequate for conclusions to be reached about the importance of temperature conditions in movements and

distribution. Observations gathered during only one season are insufficient for conclusions. It is necessary to observe the same correlations of fish movements and distribution with temperature over a number of years. If the correlations hold in spite of variations in the relations of all the environmental conditions, there is justification for concluding that it is primarily the temperature conditions that determine what the fish do.

Attention must be given particularly to temperature gradients in nature as well as to the actual temperatures in which fish are, and to other environmental conditions that could intervene in the responses of fish to temperature, before conclusions can be drawn about the rôle of temperature in determining the movements and distribution of fish in nature.

ACKNOWLEGMENT

I wish to acknowledge my indebtedness to Dr. K. C. Fisher for his painstaking and constructive criticism of the manuscript.

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